

THE ROLE OF MUCUS IN THE LOCOMOTION AND ADHESION OF THE
PULMONATE SLUG, ARIOLIMAX COLUMBIANUS.

by

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ABSTRACT

Gastropod snails move using a single appendage - the foot. For many gastropods the power of locomotion is provided by muscular waves moving along the ventral surface of the foot, the force of these waves being coupled to the substratum by a thin layer of mucus. This mucus acts as a glue which causes the animal to adhere to the surface upon which it crawls, but nonetheless allows forward movement of the animal. In fulfilling this function the mucus must show some unusual properties. This study examines the chemical and physical properties of the pedal mucus of the pulmonate slug Ariolimax columbianus, and relates these properties to the role of mucus in locomotion.

The pedal mucus of A. columbianus is a gel consisting of 96-97% water restrained by a network formed of a high molecular weight glycoprotein. The glycoprotein is a polyelectrolyte; the charged nature of the molecule causing it to swell in solutions of low ionic strengths. This swelling accounts for the glycoprotein's ability to influence a large volume of water. The gel network is stabilized both by disulfide bonds between protein moieties, and by "weak bonds" (hydrophobic interactions and/or hydrogen bonds) between glycoprotein molecules.

The physical properties of the pedal mucus were measured in shear. At shear ratios of less than 5, and over short time periods, the gel shows the properties of a viscoelastic solid. G' is 100 N/m² and $\tan \delta$ is 0.008; both

are virtually constant from 0.1 - 100 Hz. Over long periods of time the gel stress relaxes without reaching equilibrium, indicating that the weak bonds of the gel network allow the network to flow under stress.

At a shear ratio of about 5 the gel network yields as weak bonds are broken, and with further deformation the mucus acts as a viscous fluid with a viscosity of about 50 poise. If this fluid is allowed to stand unstressed, the gel network will "heal" as the weak bonds reform. This healing process begins in much less than a second and, as a result, the mucus again acts as a solid. This "yield-heal" cycle can be repeated numerous times.

These physical properties are ideally suited to gastropod locomotion. Under the moving portions of the foot (the muscular waves and the rim) the mucus is present in the form of a viscous liquid, lubricating forward motion. Under the stationary parts of the foot (the interwaves) the mucus has "healed" and, as a solid, serves as an effective adhesive. The mucus thus acts as a material ratchet allowing for effective adhesive locomotion.

The precise movements of the foot of A. columbianus were measured using a video tape recorder. The thickness of the mucus layer was measured by quickly freezing, and subsequently sectioning, crawling slugs. These data, along with the measured physical properties of pedal mucus, allow for the construction of a model predicting the forces operating under a crawling slug. This model has been tested by actually measuring these forces, and has proven accurate.

The increased oxygen consumption associated with locomotion in A. columbianus was measured as a function of crawling speed . From these data the cost and efficiency of adhesive locomotion have been calculated. Movement of A. columbianus is about ten times as costly as for a mouse of the same weight. This high cost is largely due to the cost of producing the pedal mucus. While the cost of adhesive locomotion is high, the efficiency of locomotion is roughly equal to that of a running man.

It is very likely that the measurements and predictions made in this study will apply to all terrestrial pulmonates; and it is possible that they will apply to other gastropods as well.

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CHAPTER ONE

Introduction

If you have ever been walking through the woods, or puttering in your garden, early on a summer's morning it is likely that your eye has been caught by a glimpse of reflected sunlight . If you bow to your curiosity and look closely you will see a trail of what looks like cellophane winding across the ground and at its end a slug or snail gliding along. Watching a snail or slug move, it is at first hard to imagine a simpler kind of locomotion. The animal appears to move effortlessly, as if slowly sliding downhill. However, a moment's observation and thought raise a number of difficult questions:

1. What is the source of power for this type of locomotion? As effortless as a slug or snail's movement seems, some force must be operating to propel the animal forward.

2. In general slugs and snails are herbivores. To reach the leaves that are their food they are often required to climb the vertical surfaces of plants. Watching a slug or snail climb, one is immediately struck by the fact that the animal does not change its mode of movement when it switches from crawling horizontally to vertically. Instead it seems simply to glide upwards. Obviously the animal's foot must somehow manage to adhere to the surface upon which the animal crawls. How can we account for this adhesion?

3. The adhesion of slugs and snails to the substratum

immediately raises another question: If the animal is stuck to the surface upon which it walks, how does it manage to move at all? In other words, how does an animal with only one foot contrive to walk on glue?

4. These last two questions focus attention on the slime that slugs and snails move over. That this slime is important to the animal can easily be demonstrated. Barr (1926a) has shown that the slug Milax sowerbii can neither move nor adhere without this pedal mucus. Presumably this is true of other gastropods as well. Another measure of the importance of this slime to slugs and snails is the large amount of energy they will expend in producing it. Williamson (1975) has found that between 31% and 36% of the total energy expenditure of the snail Cepea nemoralis was directed to the production of pedal mucus. What, then, is this mucus? Is it really the glue that accounts for adhesion? What are its physical and chemical properties and how do they allow for movement?

For the most part the answers to these questions are not known. While zoologists have spent a great deal of time and effort studying the flight of insects and birds, the running of quadrupeds, and the swimming of fish; many of the less flamboyant forms of locomotion, such as the adhesive locomotion of gastropods, have been largely ignored. This is unfortunate, for I believe there is much information to be gained from the study of slowly moving animals that will be of use to all biologists. In light of this belief this study was undertaken to provide answers to the questions

posed above.

I have approached the large problem of explaining this form of adhesive locomotion by examining in detail the locomotion of one animal, the slug Ariolimax columbianus. The several aspects of locomotion were studied separately: Chapters 2 and 3 describe the structure of the slug's locomotory apparatus, and the movement of this structure during locomotion. The physical properties of the pedal mucus are described in Chapter 4. Chapters 5 and 6 deal with the chemical composition and macromolecular structure of the pedal mucus. All of these results are brought together in Chapter 7 where a model is constructed to account for the locomotion of A. columbianus. The validity of this model has been tested by comparing the predicted forces of locomotion with those actually measured, and these results are also presented in Chapter 7. The model of Chapter 7 is used in Chapter 8 to examine the energetics of locomotion in slugs. Chapter 9 examines the mechanism of adhesion in A. columbianus.

This study of the locomotion of A. columbianus, while answering many questions about adhesive locomotion, raises further questions about gastropod locomotion in general. Chapter 10 discusses these unanswered questions and attempts to provide a perspective on the relationship between adhesive locomotion and the more familiar forms of animal movement.

CHAPTER TWO

Slug Morphology

Terrestrial slugs are members of the subclass Pulmonata of the gastropod molluscs . As such, their morphology is closely tied to that of the familiar garden snails from which they are considered to have evolved (Runham and Hunter, 1970). To provide a background for comparison with slug morphology, the gross morphology of snails will be reviewed here. A more thorough treatment of snail morphology may be found in Fretter and Peake, 1973.

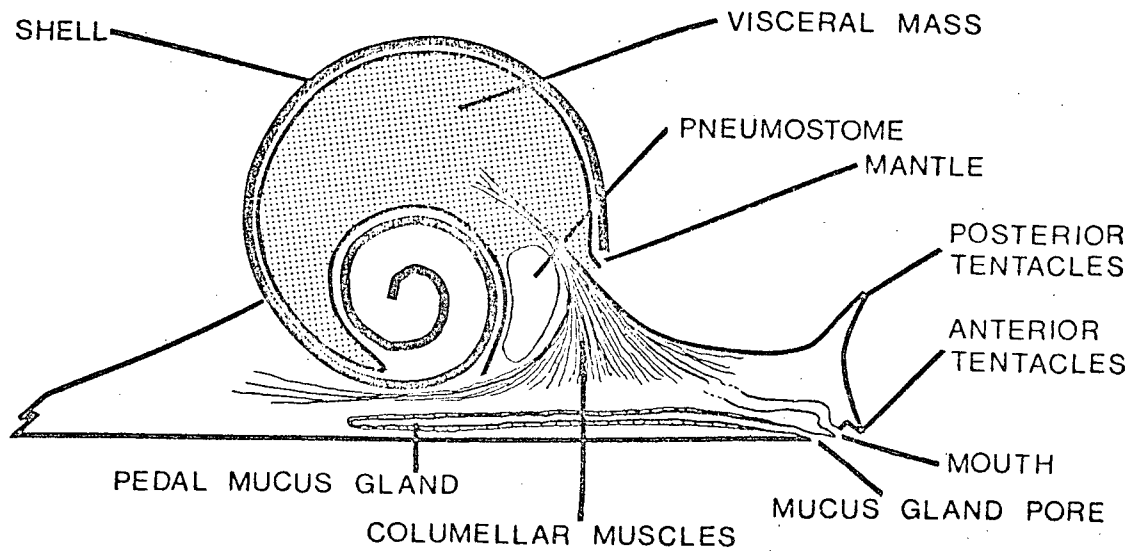
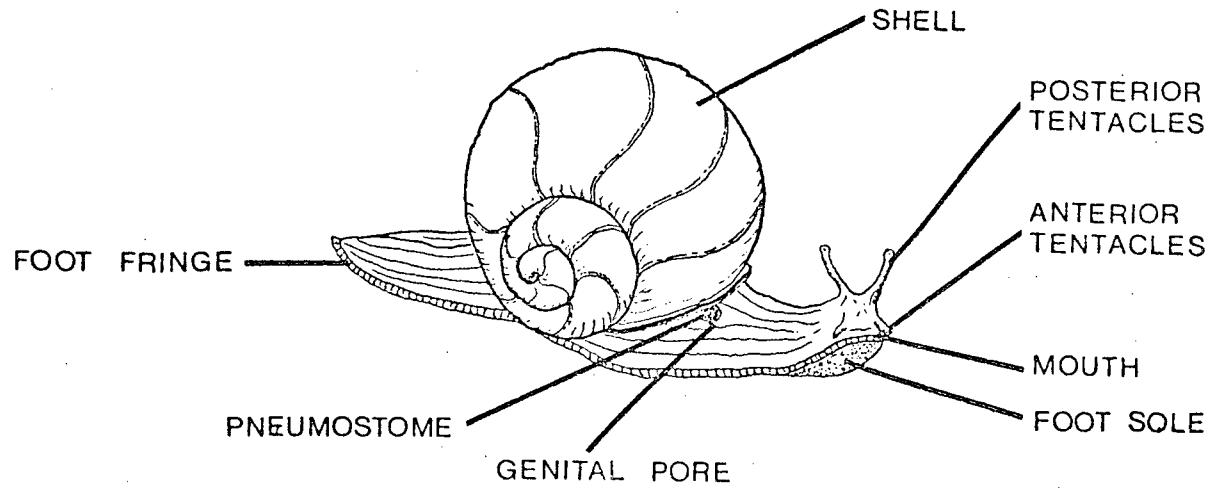
Figure 2.1 shows an idealized depiction of a terrestrial snail. The body is usually considered to consist of two parts:

1. The Visceral Mass . The internal organs of the snail are grouped together to form a compact visceral mass. This mass is enclosed within a bag-like structure, the mantle. The epithelium of the mantle secretes the materials which form the shell. The shell in turn provides support and protection for the visceral mass.

2. The Head Foot . Extending out of the shell are the portions of the body that require contact with the outside world. The foot, a hydrostatically supported, muscular structure, is suspended from the columella of the shell. Contractions and extensions of the foot provide the movements necessary for locomotion. The mucus secreted onto the foot, among other things, functions as an adhesive allowing the snail to remain anchored to the surface upon

FIGURE 2.1. A generalized gross morphology of the terrestrial pulmonate snail.

FIGURE 2.1



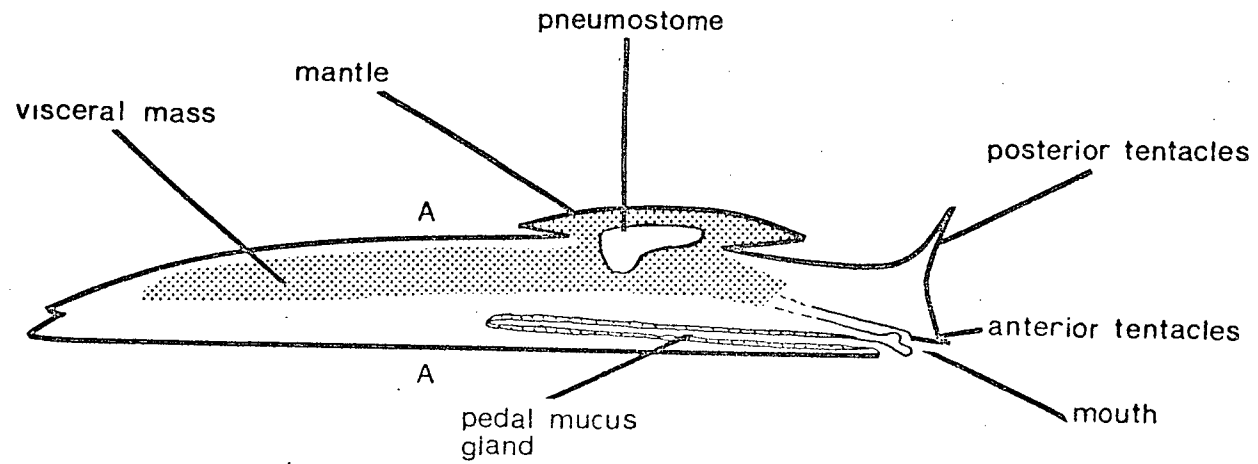
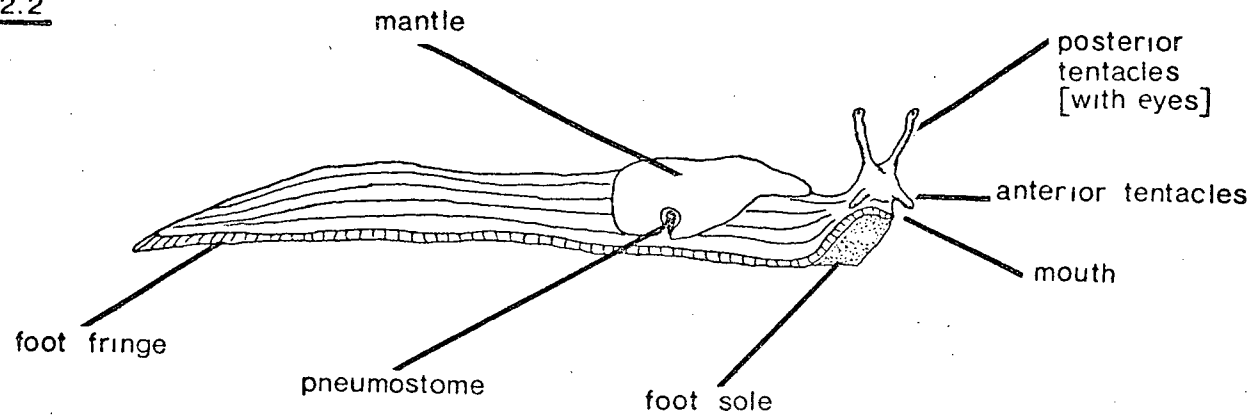
which it crawls. The foot (or pedal) mucus is produced in the suprapedal gland contained within the foot mass, and is extruded through a pore at the anterior end of the foot. The "head" of the animal consists of two pairs of tentacles and a mouth. The posterior pair of tentacles supports the eyes, the anterior tentacles are thought to serve as chemosensory organs. The mouth houses a typical gastropod rasping radula. Gas exchange occurs in a vascularized cavity (the pneumostome) opening on the right hand side of the animal behind the head. The pneumostome opening also serves as an exit for faeces and nitrogenous wastes. Snails are hermaphroditic, and both sperm and eggs are shed through structures which can be everted from the genital pore, which opens near the pneumostome. The entire head-foot can be contracted and pulled back into the shell, the shell aperture then being closed by an operculum.

The gross morphology of terrestrial slugs (Figure 2.2) is derived from that of the snail by the loss of the external shell. The visceral mass is extended to lie along the dorsal surface of the foot. The mantle, though no longer capable of producing an external shell, is still present and serves to protect the heart, kidneys, and pneumostome. In times of danger the head may also be sheltered beneath the anterior flap of the mantle. In other respects the morphology of the slug is very similar to that of the snail.

At first glance the morphology of a slug seems grossly maladapted to terrestrial existence. The slug's epithelium is highly permeable to water; and, unlike the snail, it cannot

FIGURE 2.2. The gross morphology of the terrestrial slug
Ariolimax columbianus .

FIGURE 2.2



retract into a shell to avoid desiccation. Similarly, the lack of a shell would seem to render the slug open to predation.

These disadvantages of life without a shell are apparently offset by a number of advantages (Runham and Hunter, 1970). Because it is not tied to a bulky shell a slug can fit places that a snail cannot. For example, slugs are adept at crawling under logs and into small holes in the ground where the humidity is high. The availability of such damp shelters may thus offset the lack of protection against desiccation. Slugs also have the advantage of being able to move without having to drag along a heavy shell. Finally, slugs are freed from the necessity of ingesting large amounts of calcium in order to produce and maintain a shell. The viability of these advantages is evidenced by 1) the independent evolution of the shell-less body form in at least three different families of pulmonates, and 2) the worldwide present distribution of slugs.

Slugs occur in a wide variety of sizes, from small species where the adults weigh less than a gram, to species such as the Ariolimax columbianus dealt with in this study where adults may weigh 20-25 grams.

The Foot

The foot of Ariolimax columbianus is the organ responsible for adhesion and locomotion and consequently is of prime importance in this study. To examine the fine structure of the foot several small A. columbianus were

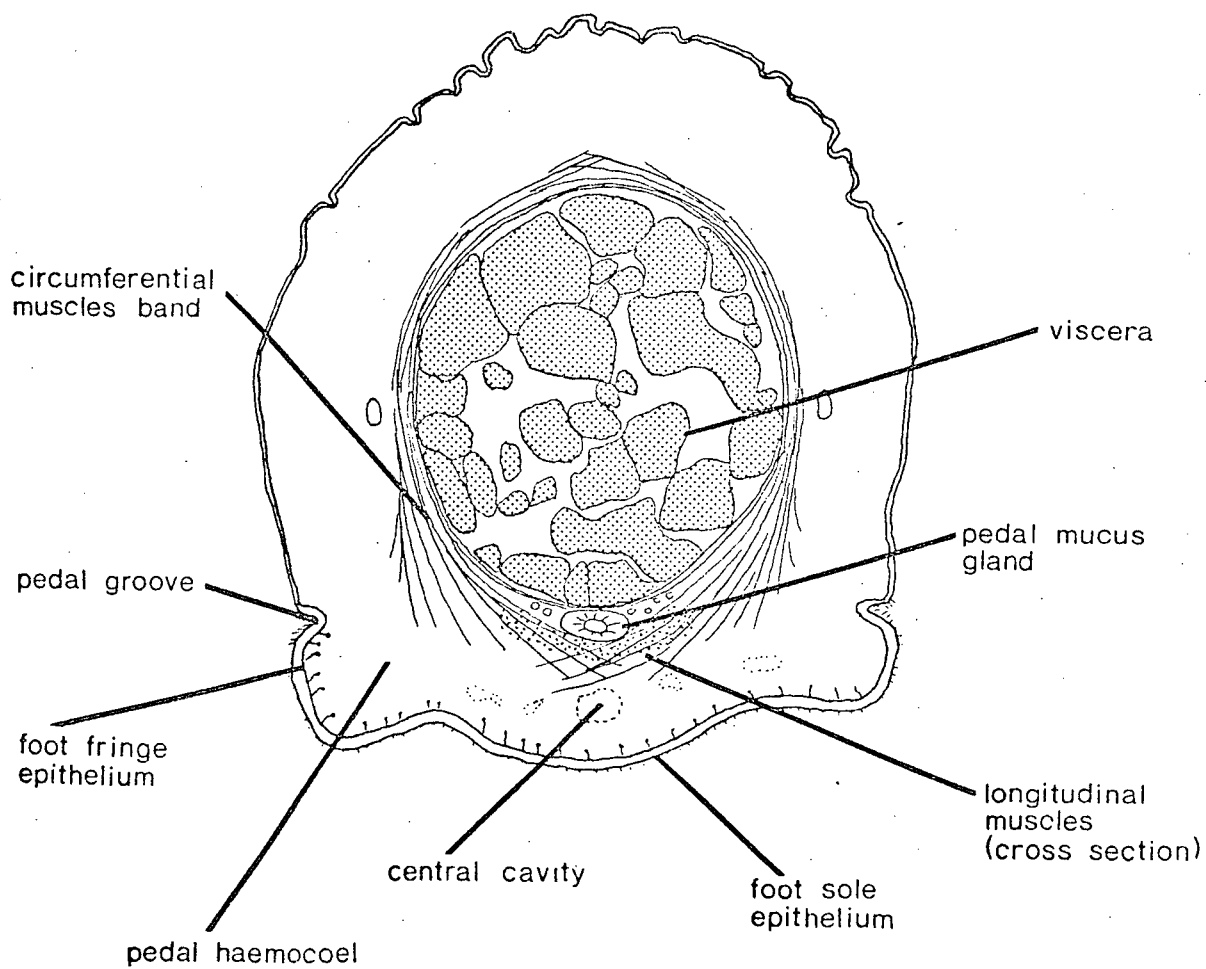
prepared for histological studies. The slugs were relaxed in water containing a small amount of MS222. The specimens were then fixed in either 1% gluteraldehyde or Bouins fixative, dehydrated, and wax embedded. Sections were cut (7-10 um) and stained either with alcian blue/eosin or by the PAS method for the detection of mucus. Sections intended for the study of muscle and connective tissues were stained with Mallory's triple stain. Figure 2.3 shows the structure of the foot. The foot consists of three structures:

1. The Suprapedal Gland. The suprapedal gland is a tubular organ embedded in the dorsal surface of the foot and extending approximately two thirds the length of the slug. Mucus is produced in the cells of the gland and secreted into the lumen. The luminal epithelium is ciliated and these cilia may assist in the movement of the mucus to the pore beneath the mouth where the mucus is extruded onto the foot sole. The structure of the suprapedal gland is very similar to that in Arion ater described by Barr (1926b).

2. The Pedal Haemocoel And Musculature. The bulk of the foot is formed of a reticulum of connective tissue and muscle fibers interspersed with haemocoelic spaces. There are two discernable large bands of muscle in the foot. The first of these is a longitudinal muscle band lying just ventral to the suprapedal gland and running the length of the slug. The second is a band running roughly circumferentially. This second band starts just proximal to one of the foot fringes and runs medially below the longitudinal muscle band. It then continues around the

FIGURE 2.3. A cross section of Ariolimax columbianus showing various structures of importance to this study. This section was made at the position indicated by the line A-A in Figure 2.2.

FIGURE 2.3



dorsal side of the slug and back down to end proximal to the opposite foot fringe. It seems likely that these two muscle bands are responsible for the large changes in body dimensions shown by these slugs. It is likely that they function in much the same manner as the longitudinal and circumferential muscles which control the shape of earthworms (Gray, 1968). A contraction of the longitudinal muscle band will cause the slug to shorten and become wider. A contraction of the circumferential muscle band will squeeze the viscera and coelomic fluid and cause the slug to lengthen. In addition to these muscle bands a dense reticulum of muscle fibers is present throughout the foot. At first these muscle fibers appear to be arranged haphazardly; however closer examination reveals some order. In a sagittal section through the foot few fibers are seen to run either directly dorso-ventrally or directly anterior-posteriorly. Instead fibers run in one of two oblique directions. Fibers originating on the pedal epithelium either run obliquely anteriorly or obliquely posteriorly. The situation appears much the same in cross-section. Few fibers seem to run either directly dorso-ventrally or laterally across the foot. This situation is very similar to that found in Agriolimax reticulatum as described by Jones (1970). The significance of this fiber arrangement will be discussed in the next chapter.

The spaces between the muscle and connective tissue fibers of the foot are filled with the haemocoelic fluid. In contrast to the situation proposed to exist in the limpet

Patella vulgata by Jones and Trueman (1973) there is no microscopical evidence to suggest that this fluid is confined by the individual spaces and therefore not free to move from one space to the next. Indeed there is a sizable cavity extending medially along the entire foot. The walls of this cavity are ill-defined and it appears to be formed of contiguous haemocoelic spaces strung together. The presence of this cavity suggests that the pedal haemocoel is structured to allow the movement of fluid along the foot. Again the significance of this arrangement will be discussed in the next chapter.

3. The Pedal Epithelium . The pedal haemocoel is bounded laterally and ventrally by the pedal epithelium. Two distinct areas are discernable in this epithelium. The epithelium of the foot fringes and the pedal groove are densely ciliated. The cilia here are quite long (about 4 μ m). Sections of this epithelium can be obtained from live slugs by careful dissection with a razor blade. The cilia continue to actively beat in this dissected tissue and their effective stroke is found to be such that mucus would be propelled posteriorly in the intact slug. Scattered mucus producing cells (the "granular" cells of Arcadi, 1963) are present on the foot fringes. The sole of the foot is covered by a second type of epithelium. Cilia are again present, but they are shorter (about 2 μ m) and more sparsely distributed. I was not able to determine the direction of the effective stroke for these cilia, however Barr (1926a,b) found that the pedal cilia in two other species of slug

transported mucus laterally and posteriorly. Scattered granular cells are also present on the epithelium of the foot sole. It has been reported by Barr (1926a,b) and Jones (1970) that in other species of slugs the mucus produced by the pedal epithelium (distinct from that produced by the suprapedal gland) is quite watery. However I have never observed the presence of watery mucous secretions on the foot of Ariolimax columbianus.

4. The Mucus The thickness of the mucus layer beneath the foot was examined using the following procedure: Small (3-4 cm length) A. columbianus were placed on a strip of aluminum foil. When the slugs were actively moving, the strip was dropped into a dewar flask containing liquid nitrogen. The slugs froze very rapidly; before they could retract their eye stalks. The procedure is similar to one developed by Lissman, (1946) and used by Jones (1973). This procedure differs from those of Lissman and Jones in that the slugs are moving over a non-porous surface when they are frozen. Lissman used a piece of copper screen and Jones a strip of filter paper.

After freezing, the specimens are fixed (1% glutaraldehyde - 50% ethanol at -20°C) for one week. After fixation specimens are dehydrated, wax embedded, and sectioned at 8-10 μ m according to standard histological techniques. Sections were stained with either Mallory's triple stain for examining the general structure of the foot, or alcian blue/eosin when the location of mucus and mucus producing cells were to be examined. Using this

technique saggital sections were examined from several slugs. In many cases the mucus layer beneath the foot had become dislodged during the process of fixation or embedding, but in those cases where the mucus layer was apparently intact it was found to be from 10 to 20 um in thickness.

With this brief description of the morphology of the locomotory apparatus of A. columbianus in mind the function of this structure may now be examined.

CHAPTER THREE

Introduction

Kinematics is the study of motion without regard for the forces that cause locomotion. As such it is a doubly appropriate term for the study of the locomotory movements of gastropods because these animals appear to glide along with no effort at all. A closer look, however, reveals a specialized set of movements that accompany locomotion, and it is a precise description of these movements that is the objective of this chapter.

The locomotory movements of gastropods fall into two broad categories. First there are those gastropods, primarily aquatic, which move by means of cilia. It has been hypothesized (Elves, 1961) that ciliary locomotion is the primitive form of movement for gastropods. Lists of species utilizing ciliary locomotion, and the various parameters of their movement are provided by Miller (1974). Most gastropods however move by means of waves generated on the ventral surface of the foot by the contraction of muscles. These muscular pedal waves are best observed by placing the animal on a glass plate and watching the foot through the glass as the animal moves. Close examination reveals a number of alternating light and dark bands (corresponding to extension or compression of the foot) which move parallel to the long axis of the foot as the animal crawls. The waves disappear when the animal stops. Presumably these pedal waves generate the reactive forces

that actually propel the animal.

A variety of wave patterns are present in gastropods, and descriptions of these pedal waves are found in several studies (Miller, 1974a, Jones, 1973, Trueman and Jones, 1970, Lissman, 1945a, and Gainey, 1976). The various sorts of pedal waves are categorized according to a scheme proposed by Vlez (1909) (as cited in Lissman, 1945a, and Miller, 1974). Waves that move in the same direction as the animal are termed direct. Waves moving in the opposite direction from the animal are retrograde. A single wave may extend across the entire width of the foot (monotaxic) or waves may be only half the width of the foot (ditaxic), alternating sides. Most species may be described by these terms, though a few unusual species have waves that move diagonally along the foot, or appear and disappear haphazardly. Ariolimax columbianus moves by means of direct, monotaxic waves.

A precise description of the movements associated with direct waves is found in Lissman (1945a) and Jones (1970) and for indirect waves in Jones and Trueman (1970). The movements of A. Columbianus observed in this study are in general agreement with these studies. Areas of disagreement will be noted as they appear in the description of A. columbianus locomotory kinematics.

A. columbianus Locomotory Kinematics (Methods)

Ariolimax columbianus were collected in the woods near the University of British Columbia. Slugs were kept at 10

°C in a controlled environment chamber. The lights of the chamber were cycled, 12 hours of light alternating with 12 hours of darkness. The slugs were fed lettuce and carrots. Under these conditions slugs could be kept healthy for up to a year. All tests conducted on live slugs reported in this study were carried out at room temperature (21-23 °C). Slugs brought into the laboratory from the cold room were allowed to come to room temperature before tests were conducted.

The locomotory movements of A. columbianus were observed by allowing a slug to crawl on a glass plate. Movements of the ventral surface of the foot were recorded with a Sony video tape recorder at 60 frames/second. Taped records were played back onto a 25 inch (diagonal) television screen. When desired, the tape could be analyzed frame by frame. A ruler taped to the glass plate near the slug allowed for the size calibration of images on the television screen.

A stationary slug shows no evidence of pedal waves. The foot is dark tan, the color darkest near the center of the foot where the dark digestive gland shows through the thin layer of pedal musculature. As the slug begins to move, waves first appear 1/4 to 1/3 of the foot length back from the anterior end of the foot. When the pedal epithelium and musculature are compressed in the wave they mask the dark digestive gland. Consequently, a wave of contraction appears lighter than the resting foot. As these waves move forward the area of wave formation moves

posteriorly until waves are present along the entire foot as shown in Figure 3.1. Thirteen to 17 waves are present on the foot. The pattern of waves occupies only the central $1/3$ to $1/2$ of the foot, waveless areas forming a "rim".

The overall movements during locomotion are best understood by following a single wave in a schematic diagram (Figure 3.2a). The wave is formed near the posterior end of the foot, presumably by the contraction of a segment of the pedal musculature. This contraction causes an area of the foot to compress antero-posteriorly to some fraction of its relaxed length. As a consequence of this contraction the tail moves forward a bit. From video recordings of pigmented areas on the foot, it is possible to measure the extent of this compression in a crawling A. columbianus. Such measurements were made on three slugs, three to four waves being measured on each slug. As a result of these measurements it was found that for A. columbianus the ratio of (extended length/compressed length), the compression ratio, ranges from 1.43 to 2.03 with an average of 1.69.

This wave of compression is passed forward along the foot as muscles ahead of the wave contract and muscles behind relax. When the wave reaches the anterior end of the foot, muscles are no longer available to keep the wave compressed and it returns to its resting length. The energy for this re-expansion is presumably provided by hydrostatic pressure within the foot and will be discussed further later in this chapter. As a consequence of the re-expansion of this segment of the foot, the anterior end of the foot is

FIGURE 3.1. A schematic representation of the ventral surface of the foot of a typical Ariolimax columbianus , showing the relative positions, movements, and areas of the rims, waves, and interwaves.

Figure 3.1

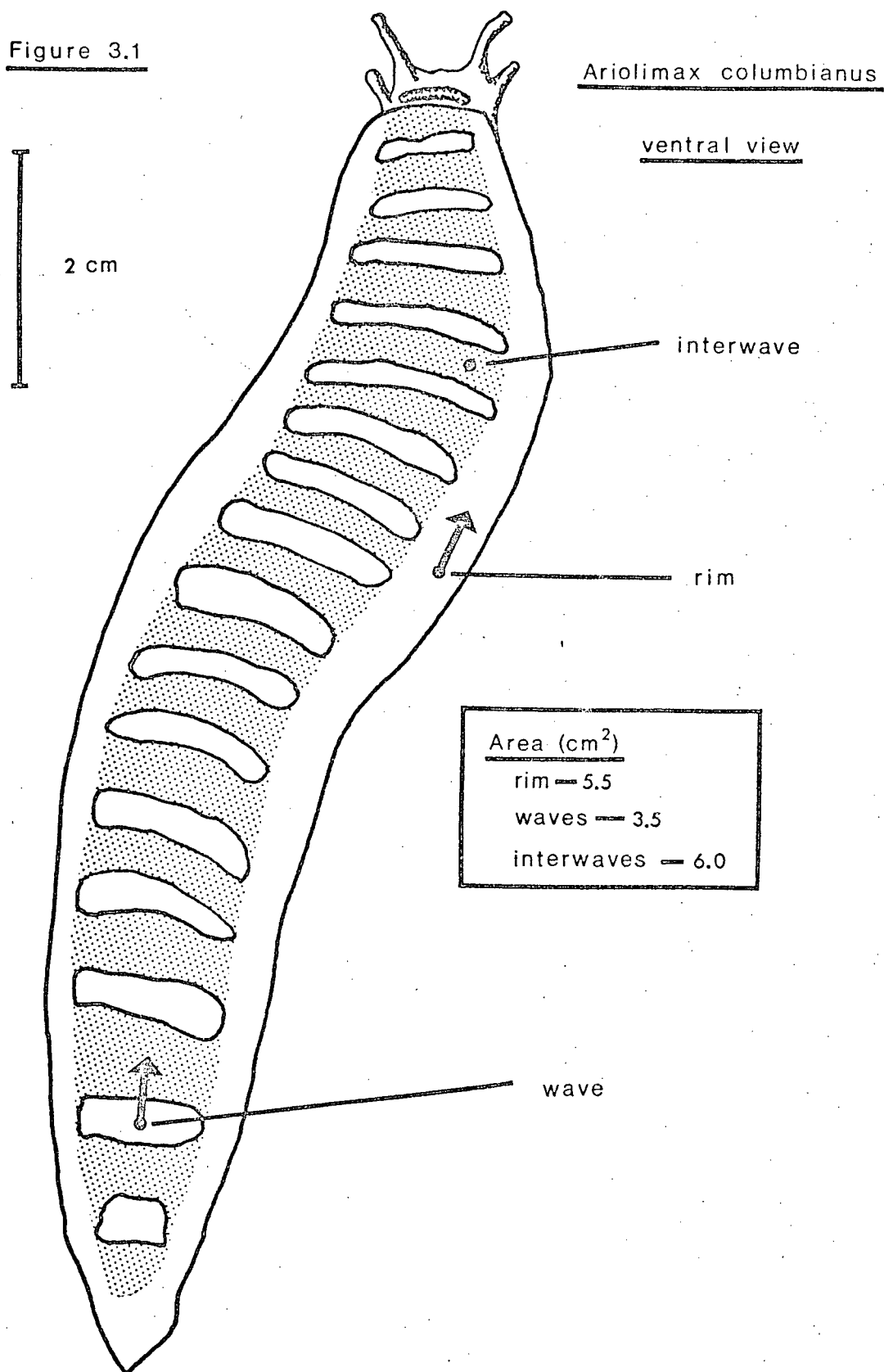
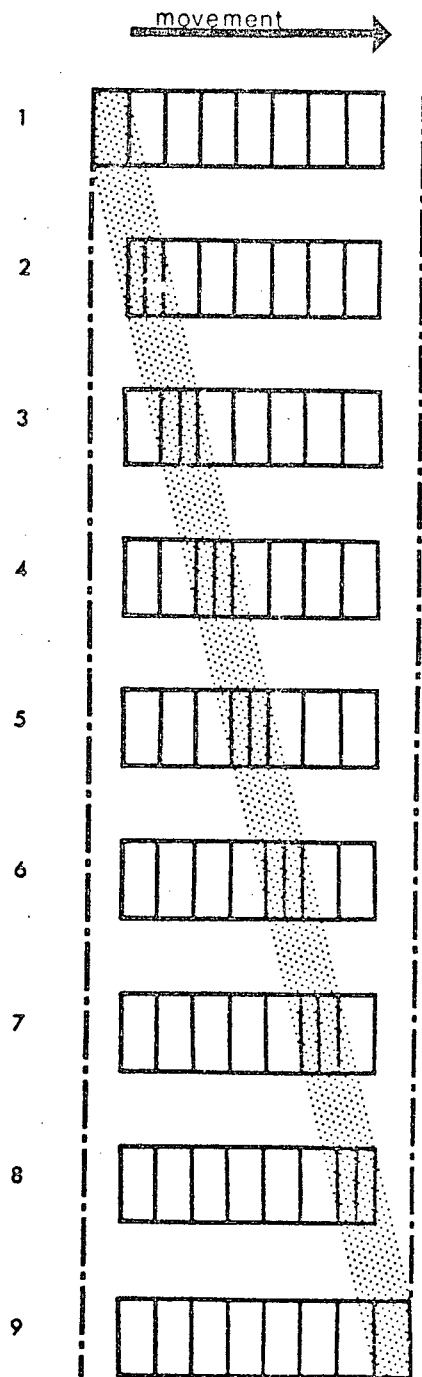
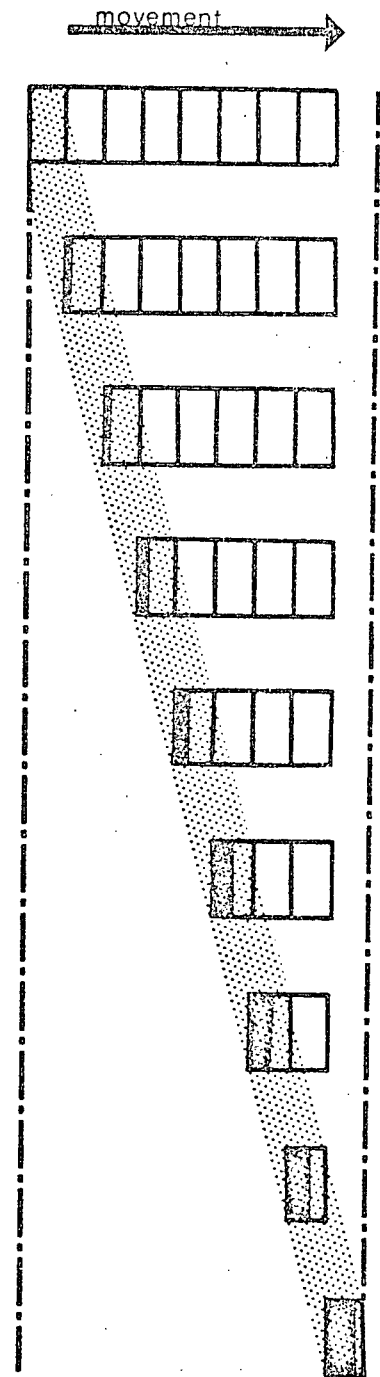


FIGURE 3.2. A diagrammatic explanation of the mechanism by which a wave of compression can result in movement.

- A) As the wave moves from left to right (shown by the stippled line) the foot as a whole is transported to the right.
- B) The movement of the foot at the same speed as the wave results in the collapse of the foot.

Figure 3.2

AB

moved forward. Thus, each wave constitutes one "step"; the distance the tail is pulled forward when the wave forms is transferred to the head when the wave of compression re-expands. The distance A. Columbianus advances as a consequence of each wave is about 1.0 to 1.5 mm.

Notice in Figure 3.2a that those parts of the foot which are not contained in a pedal wave are stationary relative to the ground. Only when a portion of the foot is contained in the wave of compression is it moving.

One further point must be explained here. This involves the difference between the speed at which a wave travels forward along the foot and the speed at which the segments of the foot move as they are momentarily contained in a wave (hereafter known as the segment speed). This difference is somewhat confusing and is perhaps best explained by analogy.

Imagine a group of surfers who have arranged themselves in the surf along a line perpendicular to the beach. Thus the first surfer is farthest from the beach, the second surfer is just shoreward of him, and so on, each surfer waiting to catch a wave. As a wave comes in the first surfer tries to catch it, is accelerated forwards a bit, but never gets up to speed and the wave passes him by. The second surfer does likewise and so on down the group as the wave moves into shore. After the wave has passed, each surfer will have moved shorewards slightly but the linear arrangement and order will be the same. Now a second wave arrives. This time the first surfer "catches" the wave,

ie. he begins to move at the same speed as the wave. The wave and first surfer advance on the second surfer who also catches the wave and so on down the line. When the wave reaches the last surfer all the surfers will be travelling forward at the same speed and since all are on the same wave all will reach the shore at the same time. This can only happen if the linear arrangement of the surfers collapses as the wave travels down the line. If each surfer catches the wave the linear arrangement can only be maintained if the surfers are compelled to decelerate and hop off the wave before they reach the next surfer in line.

Now apply this analogy to the schematic drawing of 3.2b. Imagine that as the wave of compression moves forward, the compressed segments begin to travel at the same speed as the wave. As the wave advances, more and more compressed segments are added, further compressing the segments already moving forward. The final outcome is that all the segments of the foot must arrive at the anterior end of the foot at the same time. This is a physical impossibility. This situation can be avoided in reality by two methods: 1) either the segments of the foot never move with a speed equal to the wave speed or 2) segments on the foot that momentarily move at the wave speed are compelled to decelerate by the physical constraints of the foot structure, ie. the finite extensibility and compressibility of the foot. As a consequence each segment is left behind by the wave after it has travelled forwards a certain distance and the linear arrangement of segments on the foot

is maintained (Figure 3.2a). Thus the wave speed may be equal to, but is usually greater than, the segment speed.

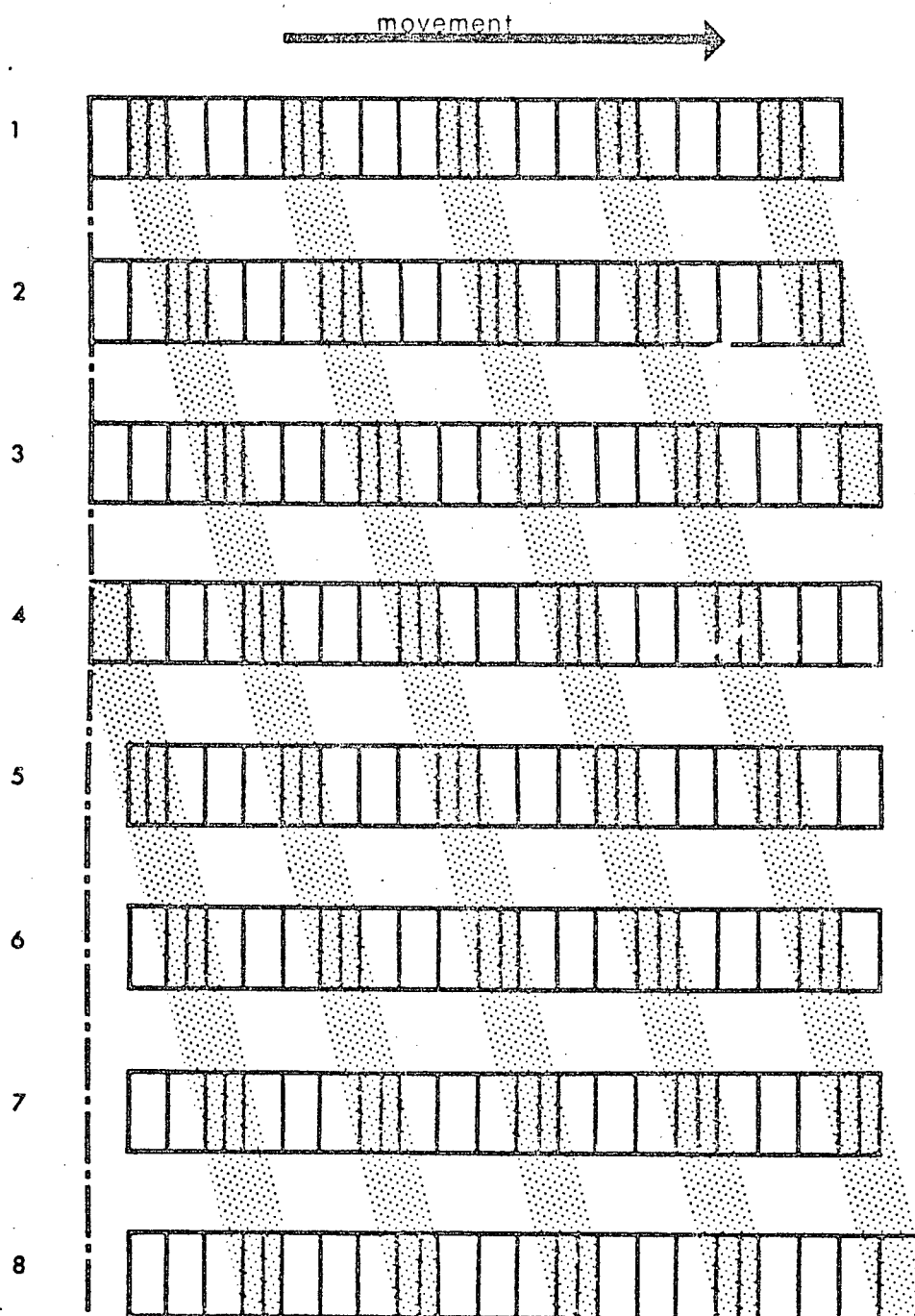
Figure 3.3 is a schematic representation of the foot of a slug when several waves are present. It can be seen that the presence of multiple waves does not change the basic sequence of movements. Again the foot moves forward only when compressed in a wave; the area between waves (the interwaves) are stationary relative to the ground.

The schematic diagrams of Figures 3.2 and 3.3 describe the situation that exists in the central portion of the foot of a moving slug. How is the rest of the slug tied to this part of the foot, and how does this explanation account for the apparently continuous forward movement of a slug? The answer to both these questions can be explained by an analogy to a more familiar mode of locomotion: The torso of a running person moves forward at a constant speed relative to the ground. His feet however are constantly changing velocity. Obviously when a foot is planted on the ground it is stationary relative to the ground. When the foot is lifted from the ground it travels forward with a velocity greater than that of the torso. This velocity eventually carries the foot ahead of the torso at which point the foot is planted and the process is repeated. The speed of the torso is equal to the average speed of the feet. This in turn is equal to the number of steps taken per second times the length of each step.

This analogy can be transferred directly to the slug. The slug's "feet" are the series of waves and interwaves. A

FIGURE 3.3. A diagrammatic explanation of the mechanism by which several waves of compression can exist on one foot and result in movement. As the waves move from left to right the whole foot is transported to the right.

Figure 3.3



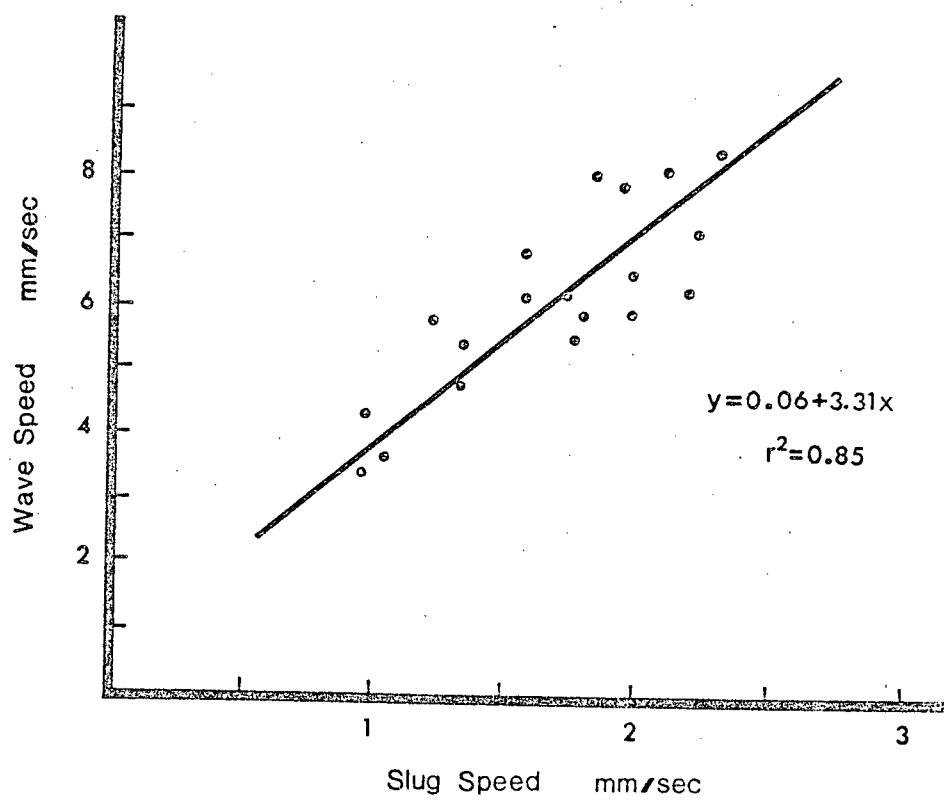
segment of the foot is alternately stationary (in an interwave) and moving forward with a velocity greater than that of the slug. The average forward velocity of the central portion of the foot will thus be equal to the constant velocity of the slug as a whole. This in turn is equal to the number of waves reaching the front of the slug per second (the stepping rate) times the distance gained by the re-extension accompanying each wave (step length, a function of the compression ratio). The fact that there are 13-17 waves present on the foot ensures that waves will constantly be reaching the anterior end of the foot. Consequently the slug moves at a constant rate. This constant rate applies to all portions of the slug not directly associated with pedal waves, including the rims of the foot (which do not develop pedal waves).

Since the average speed of the central portion of the foot must equal the speed of the whole slug, the speed of moving segments of the foot will depend on the relative periods of time spent moving and stationary. Measurements from 22 video recordings of crawling A. columbianus show that points on the foot spend roughly equal periods of time in and out of pedal waves. Consequently the average speed of a segment in a wave must be approximately twice that of the whole slug.

Video recordings also allow for a measurement of wave speed relative to the speed of the whole slug. Figure 3.4 is a plot of wave speed and slug speed for 18 measurements from 8 slugs. The ratio of wave speed to slug speed is

FIGURE 3.4. The relation between the speed of the pedal waves and the overall speed of the slug. The waves move more than three times as fast as the slug.

Figure 3.4



about 3.3.

This knowledge of wave speed and segment speed provides a check on the value of compression ratio cited earlier. Since wave speed is 3.3 times the whole slug speed, a wave will advance into a stationary interwave at 3.3 times the slug speed. Segments moving in a wave, however, are themselves travelling at 2 times the slug speed, or at a speed relative to the wave of $3.3/2=1.65$. Since the segments in a wave move slower relative to the wave than segments in an interwave, the antero-posterior dimension of a wave segment must be less than an interwave segment by a ratio 1.65 if equal time is to be spent moving and stationary. The calculated compression ratio is thus 1.65, which compares closely to the measured average of 1.69.

The information contained in Figure 3.4 may be put to further use; helping to explain the manner in which slugs control the speed at which they walk. While no slug could be said to move very fast, it can be seen from Figure 3.4 that individuals are capable of a range of speeds from slightly more than 1mm/sec to slightly more than 2 mm/sec. The slug could control its speed in one of three ways:

1. The slug could vary the number of waves present. All other factors remaining constant, increasing the number of waves present on the foot would increase the slug's speed. In the course of measuring the wave and slug speeds for Figure 3.4, the number of waves present on the foot of the crawling slug were counted. While the number of waves present varied from slug to slug, the number of waves

present on the foot of an individual did not vary, regardless of speed.

2. The slug could vary the compression ratio of the waves. Increasing the compression ratio will increase the step length and thereby increase speed without effecting any other wave factor. If this method were present in reality, the wave speed should be independent of slug speed. Figure 3.4 shows that this is not so, leading to the final possibility.

3. The slug could vary the wave speed. Increasing the wave speed, all other factors remaining constant, will increase the stepping rate and thereby the slug speed. As Figure 3.4 shows, this is indeed the case. Thus it appears that A. columbianus controls the speed of its movement by controlling the rate at which waves travel along the foot. These observations are very similar to those of Crozier and Pilz (1924) who were working with Limax maximus. Crozier and Pilz, however, found an increase in step length with increasing speed, though this effect was less pronounced than the increase in wave speed.

The observations reported above for A. columbianus are an accurate description of the gross movements of the foot during locomotion. It will be useful however to know the precise movement of individual points on the foot. To this end two procedures were carried out.

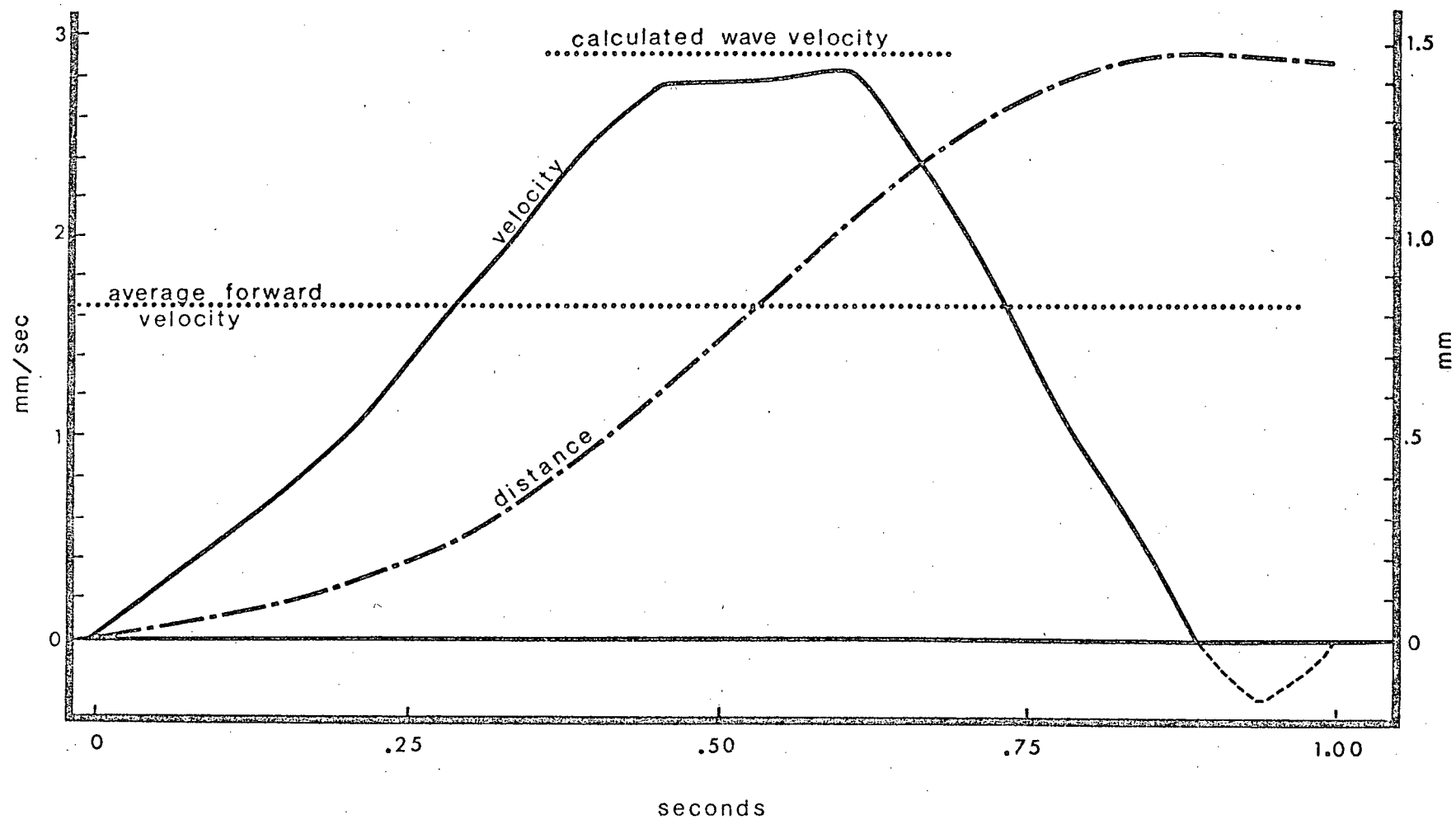
1. Video tapes were made of the foot of a moving slug while the television camera was mounted on a dissecting microscope. Magnification was such that a 4 mm length of

foot occupied the entire vertical dimension of the television screen. At this magnification individual blemishes and concentrations of mucus glands are visible on the foot and their movements can be recorded. The spatial resolution of the system at this magnification is about 20-30 μm . This factor is controlled primarily by the "jitter" of the image on the television screen. As a consequence of this jitter a strict frame by frame analysis is not possible. It is necessary that a number of frames, 5-10, be examined before it can be stated with any certainty that a point has moved. Thus, while a movement of 20-30 μm can be detected between the start and finish of a 5-10 frame segment, the time within this segment when the movement actually occurred cannot be specified. Spatial resolution is thus gained at the expense of temporal resolution.

Figure 3.5 shows an average velocity profile for a point on the foot during the passage of a pedal wave. It can be seen that as a wave overtakes a point on the foot, that point is gradually accelerated to a peak velocity. Notice that this peak velocity is considerably greater than the average velocity but is still less than the wave velocity. The peak velocity is maintained for a short period before the point is decelerated back to zero velocity. It is often found that deceleration continues past zero; in other words there is some backslip after the wave has passed. This backslip is small, amounting at most to about 30 to 50 μm . As a consequence of this small amount of backslip the time course of backslip is uncertain, as

FIGURE 3.5. The velocity and distance profiles of an average pedal wave. The curves represent values averaged from 22 separate crawls by two Ariolimax columbianus.

Figure 3.5



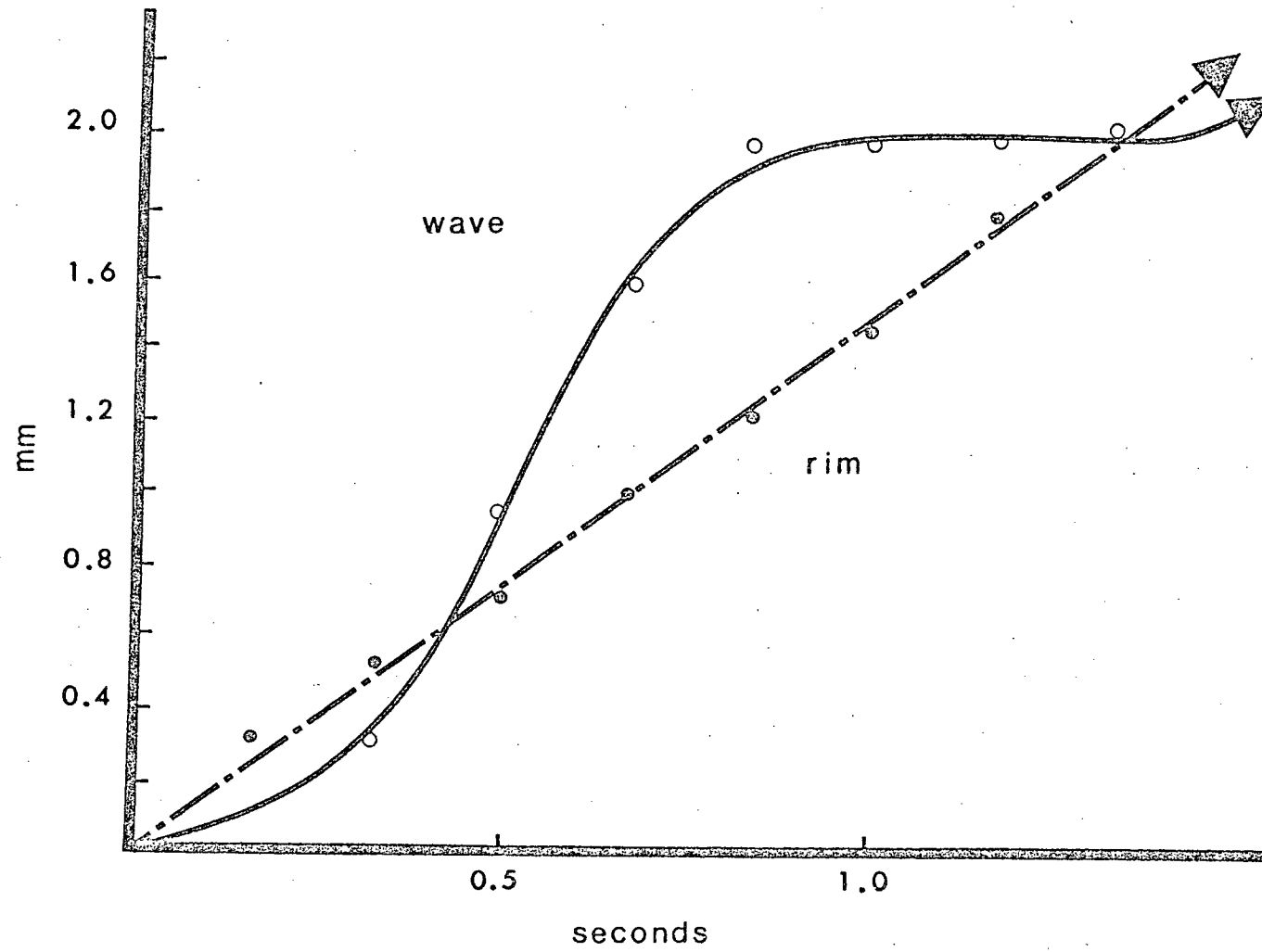
explained above. It is certain however that it happens in the first $1/6$ of a second after zero velocity is reached.

By positioning the television camera correctly it is possible to record the movement of points in the central area of the foot and adjacent points in the rim simultaneously. The results of one such measurement are shown in Figure 3.6 and confirm the observations made on the whole foot. The measurement is started just as the wave reaches the point in the central portion of the foot. At this time the central and rim points are adjacent. The central point accelerates rapidly forward, soon reaching a velocity such that it gains ground on the rim point. The point in the rim moves at a constant speed. The distance gained by the central point is just sufficient so that the rim and central points are again adjacent as another wave overtakes the central point.

2. Video recordings provide accurate measurements of motion parallel to the plane of the glass plate on which the slug crawls. They do not, however, provide information about movements perpendicular to this plane. To investigate the possible dorso-ventral movements of the foot during locomotion the slugs were quickly frozen and sectioned as described in Chapter 2. In saggital or parasaggital sections of the foot the pedal waves can be clearly seen as shown in plate 3.1. The evidence provided by these sections suggests that the slug does not lift the foot during the passage of a pedal wave when walking on a non-porous substratum. Instead, the pedal wave consists entirely of an

FIGURE 3.6. The continuous forward movement of the rims and the alternate movement and non-movement of the center of the foot result in the same average forward velocity.

Figure 3.6



- PLATE 3.1. a micrograph showing the pedal epithelium of a crawling slug.
- A) A sagittal section showing the transition between a wave (W) and an interwave (I). Notice that the foot is not lifted in the wave.
- B) A higher magnification of the epithelium under a wave. The epithelial cells are compressed antero-posteriorly.
- C) A higher magnification of the epithelium under an interwave. The epithelial cells are extended antero-posteriorly.

Plate 3.I



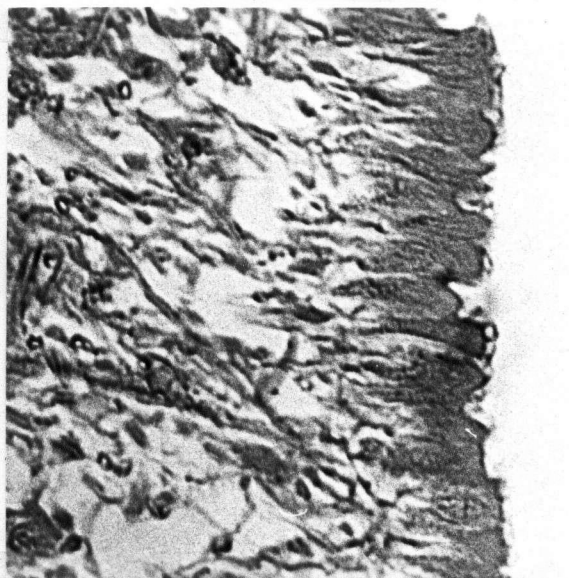
A

W

25 um

I

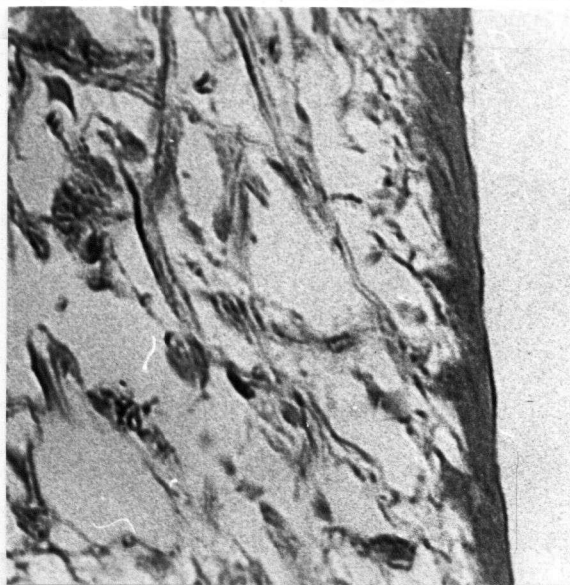
B



W

20 um

C



I

20 um

anterio-posterior compression. This can be seen by examining the shape of epithelial cells. In the extended portion of the foot the cells are long antero-posteriorly and short dorso-ventrally. These dimensions are reversed in the compressed areas of the foot. The compression ratio measured on the section shown in Plate 3.1 is about 2, in rough agreement with values calculated by other methods.

These results differ from those of Lissman (1945a) working with Helix aspersa and Jones (1973) working with Agriolimax reticulatus. Both of these authors found that the foot is lifted as it is compressed in a pedal wave. It is possible that this difference in the shape of pedal waves simply reflects a species difference. Another explanation, however, appears more probable. Explanations of gastropod locomotion that require the foot to be lifted have suffered from three major problems, all related to the mucus sandwiched between the slug and substratum (see Figure 3.7).

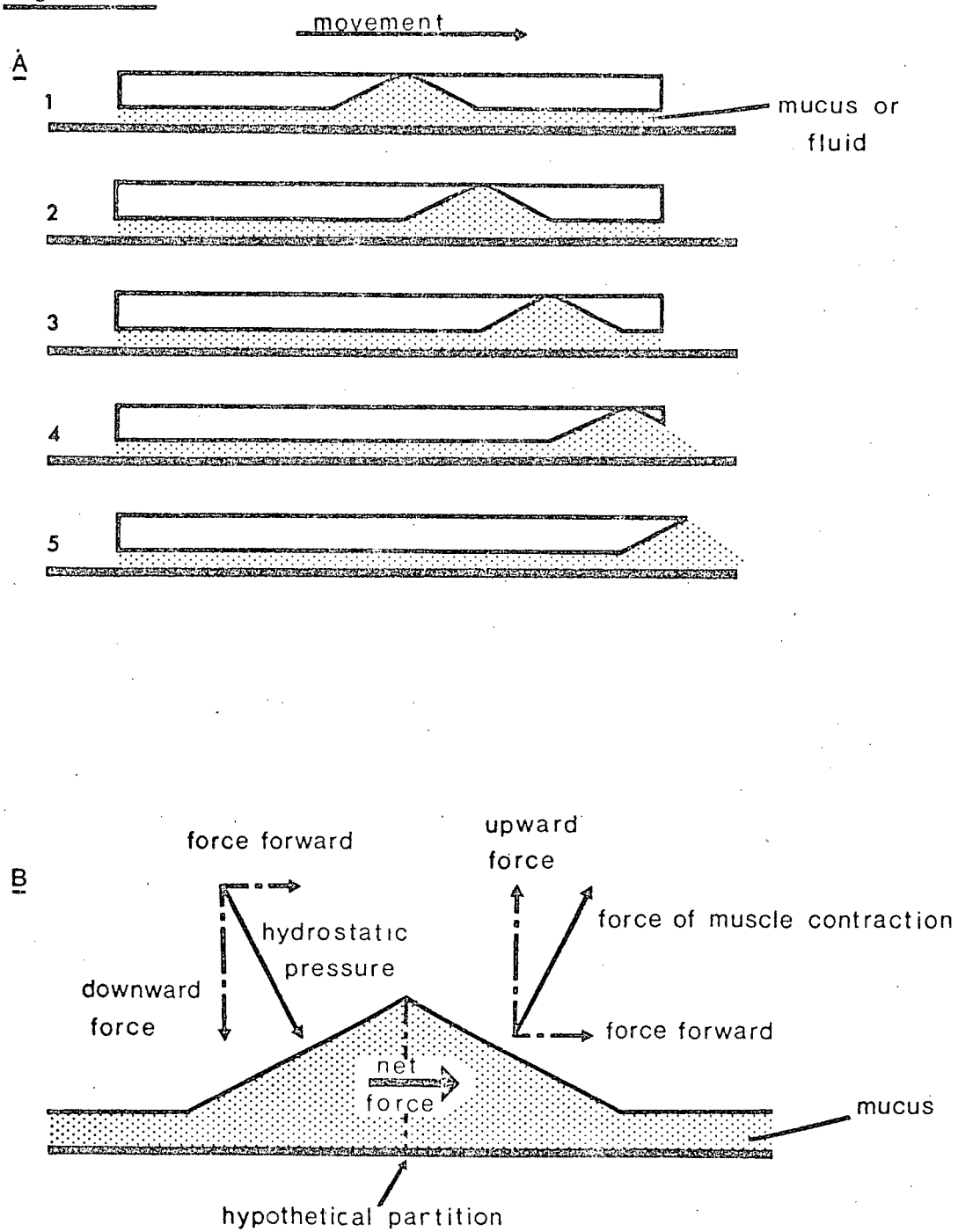
1. It has been assumed (Lissman, 1945a; Jones, 1973; and Jones and Trueman, 1970) that when the foot is lifted the space beneath the lifted portion is filled with either mucus or some fluid exudate. As the wave travels forward, this volume of mucus or fluid travels with it and should be deposited in front of the foot. This process has never been observed. Jones (1973) speculates, but without evidence, that this mucus or fluid is somehow reabsorbed into the foot.

2. Once the foot is lifted in a wave it must be returned to the substratum to which it will adhere during

FIGURE 3.7. Problems with lifted pedal waves.

- A) A lifted wave transports mucus or fluid to the anterior end of the foot.
- B) Unless a partition were to separate the two halves of the lifted wave, the downward force of the hydrostatic pressure would counteract the upward force due to muscular contraction, leaving only a net forward force.

Figure 3.7



the interwave. Mechanisms proposed to date (Lissman, 1945a; Jones, 1973; Jones and Trueman, 1970) to account for the lifting and subsequent lowering of the foot require two processes to occur simultaneously: 1) The contraction of the muscles which raise the foot pulls upwards on the mucus beneath a wave, creating an area of low pressure (see Figure 3.7b). It is this predicted low pressure which would pull fluid out of the foot to fill the space beneath the wave. 2) At the same time the hydrostatic pressure within the foot (supposedly responsible for forcing the foot back to the substratum) is pushing downwards on the mucus beneath the wave creating an area of high pressure. However, unless a rigid partition (as shown in Figure 3.7b) separates the two halves of the wave, the mucus beneath the wave must all be at the same pressure. In other words if no partition exists (and none has been found) the downwards and upwards forces will cancel and the mucus will be at ambient pressure. Jones and Trueman (1970) however measure a substantial negative pressure (ie. an upwards force) under pedal waves of the limpet Patella vulgata .

3. It can also be calculated that a very large force would be necessary to lift the foot from the substratum as the animal first begins to move. For the foot to be lifted fluid must flow into the new volume created, either from the surrounding mucus or from the tissues, and this flow must occur in the time course of a single wave (about 1 sec). Such flow would require the application of upwards stresses on the foot on the order of 10,000 kg/cm². This factor is

fully explained in Chapter 9.

As a consequence of the problems described here these mechanisms which require lifting of the foot are less than convincing.

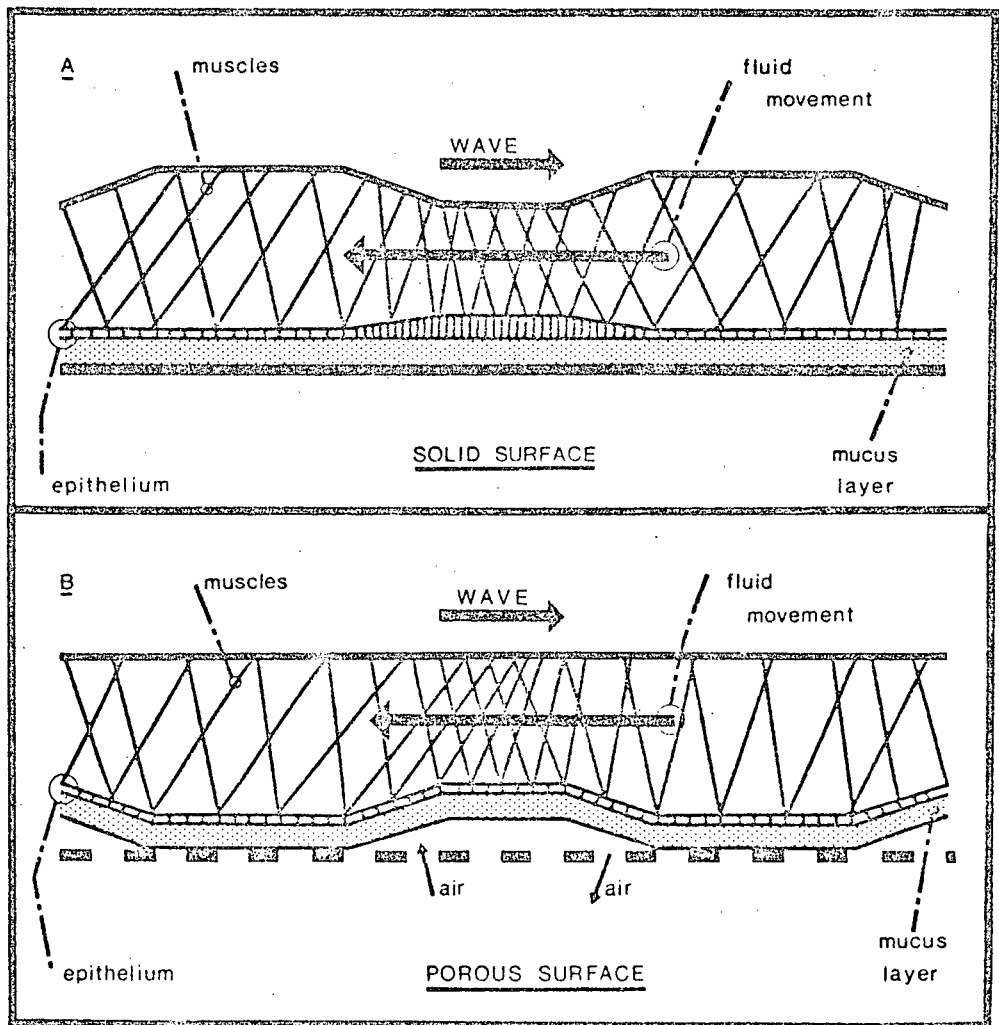
Mechanism For Slug Kinematics

The results of this study suggest a mechanism that both avoids these problems and explains the results of the previous authors. It is essentially a minor change in the mechanism proposed by Jones (1973). Simply stated, this change requires that a slug will not lift its foot during locomotion if it is crawling on a non-porous, inflexible surface. Rather, the mucus layer remains constant in thickness and the mucus is sheared between the moving foot and the stationary substratum. However, during movement on a porous surface it will lift its foot.

This mechanism is shown schematically in Figure 3.8. When the animal is walking on a nonporous surface the properties of the mucus beneath the foot are such that the muscles and connective tissue support of the foot are incapable of lifting the foot from the substratum. This assumption concerning the properties of the pedal mucus will be substantiated in Chapter 9. Unable to lift the foot, the oblique muscles instead act to pull the body cavity down thereby narrowing the spaces of the pedal hemocoel. The force required to pull the body cavity down is counteracted by a force pulling upwards on the mucus beneath the foot. This upwards force will appear as a negative pressure in the

FIGURE 3.8. . A model for the movement of the foot of Ariolimax columbianus on a solid substratum (A) and a porous or flexible substratum (B). The forward movement of the wave and the decreased thickness of the foot result in a high hydrostatic pressure in the haemocoel ahead of the wave.

Figure 3.8



mucus to a sensor on the substratum surface. Again, however, this upwards force is not accompanied by dorsally directed movement of the foot. As the wave moves forward the fluid in the pedal haemocoel is forced to flow through the narrowed channels in the area of muscular contraction. This raises the pressure of the haemocoelic fluid ahead of the wave. Since the pressure required to force fluid through a tube at a given rate is an inverse fourth power function of radius, it is only necessary for the spaces of the haemocoel to be narrowed slightly to create a substantial pressure in the haemocoel ahead of the wave. It seems likely that this mechanism (rather than contraction of the ventricle as suggested by Jones (1973) is responsible for the hydrostatic pressure of the haemocoel. This situation is analogous to the peristaltic pumping of fluid through a tube. It is this haemocoelic hydrostatic pressure that is responsible for re-extending the foot at its anterior end.

This mechanism does not require that mucus or fluid beneath the foot be transported forward with each wave, and since the foot is never lifted there is no problem with getting it back down. It also accounts for the measurement of negative pressures under the pedal waves.

When the slug is walking on a porous or flexible surface the situation is changed only in that the oblique muscles are now capable of either pulling the foot away from the substratum or deforming the substratum itself. In the case of a porous surface the ability to detach the foot is a

consequence of the porosity of the surface which allows air to easily replace the displaced mucus. It is much easier for air to move into this space than it would be for mucus. The lowering of the foot is accomplished by the hydrostatic pressure in the pedal hemocoel, created as explained above. In order for the foot to be forced down only the air beneath the wave must be displaced, a process again facilitated by the porosity of the surface. In nature slugs are required to walk over surfaces of many types. While many of these are porous or flexible there are smooth, nonporous, inflexible structures such as the bark of some trees and shrubs, the stalks of plants, and the surfaces of rocks on which the slug would not be able to lift its foot.

At present this mechanism is primarily speculation. It will require further experimentation before it can be substantiated.

One further phenomenon may be mentioned here. If a slug is lifted off the substratum and held in mid-air, it will attempt to crawl. Pedal waves will move anteriorly on the foot and, if some chalk is dusted on the foot, the mucus layer can be seen to move posteriorly. If a straight chalk line is drawn laterally across the foot it can be seen that the mucus in the central portion of the foot and that along the extreme edges of the foot move posteriorly faster than mucus in the rims. Consequently the chalk line is deformed to the shape of an "M". It is difficult to imagine a mechanism whereby the pedal waves can account for this movement of mucus. It seems much more likely that the

movements of cilia, as described in Chapter 2, are responsible . However, Litt et al. (1976) have found that the effectiveness with which mucus is transported by cilia is dependent on its storage modulus. They found that mucus with a G' of $.1 \text{ N/m}^2$ was transported most effectively by the ciliated epithelium of a frog palate. A. columbianus pedal mucus, with a G' of 100 N/m^2 , would, by this criterion, not be effectively transported. Beyond these general observations this phenomenon has not been investigated.

CHAPTER FOUR

Physical Properties

The importance of pedal mucus in the locomotion and adhesion of gastropods has been known for a considerable period of time. Barr (1926a) reports that slugs deprived of a supply of pedal mucus by the cauterizing of the pedal gland can neither walk nor adhere. Later authors (Lissman, 1945b; Jones, 1973) remark on the obvious importance of pedal mucus to locomotion. However none of these authors, nor any other author to my knowledge, have conducted tests to ascertain the properties of pedal mucus.

It will be shown in this chapter that A. columbianus pedal mucus is an unusual viscoelastic material. Before describing the physical properties of this slug's slime it will be useful to review the basic concepts, terminology, and testing procedures used in the study of viscoelastic materials.

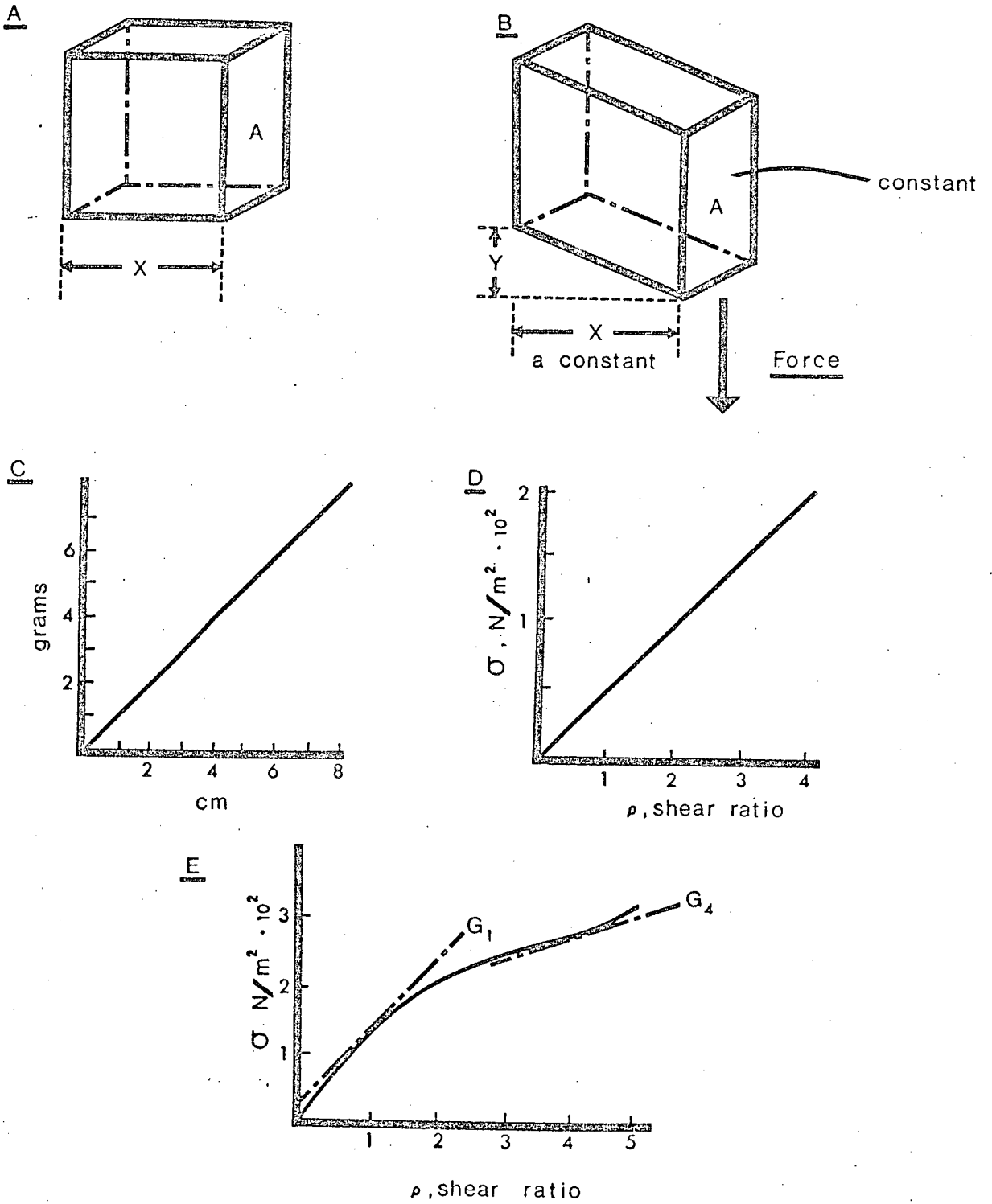
The term viscoelastic is used to describe a material that simultaneously shows properties typical of both fluids and solids: solids are elastic, fluids are viscous. Elasticity and viscosity are precisely defined concepts.

Elasticity

The primary characteristic of solids is that when pushed on they push back. This concept is quantified in the term elasticity. Take as an example the cube of material shown in Fig. 4.1a. Imagine that one side of the cube is glued to a non-moveable structure and the opposite side is

FIGURE 4.1. The properties of elastic solids. An undeformed cube (A) is deformed (B) by the application of a force. Force (or weight) can be plotted against deformation (C). Normalizing force and deformation to the dimensions of the sample allow (C) to be replotted (D) in terms of stress (force/area) and shear ratio (Y/X). The slope of the stress/shear ratio line is the shear modulus, G . The stress/shear ratio curve need not be linear, as shown in (D), but G at a point is still the slope of the line at that point.

Figure 4.1



glued to a plate on which one can hang weights. With no weights applied the cube will be undeformed. When a small weight is hung from the plate the cube will deform a small distance as shown in Fig. 4.1b. If the weight is removed the cube will immediately revert to its undeformed shape. A larger weight applied will cause the cube to deform a larger distance, and again upon removal of the weight, the deformation will disappear. By adding weights of increasing size and recording the deformation at each weight a curve such as Fig. 4.1c can be drawn. This curve of weight versus deformation refers to the particular cube, a piece of the same material of different shape or size will require different weights to achieve the same deformation. In order to describe the properties of the material from which a sample is made rather than the shape of the sample, weights and deformations are normalized. A weight hung on the plate in Fig. 4.1a places a known force on the cube and this force is distributed across the area of the cube face. This particular force and area can be normalized by dividing one by the other (force / area) to arrive at a term called stress (σ). The deformation incurred by this stress can be normalized to the dimensions of the sample, in this case the sample's thickness. The ratio (deformation/thickness) is known as the shear ratio symbolized by ρ . For small deformations the shear ratio is approximately equal to the shear strain ($\tan \alpha$ in Fig. 4.1b). For large deformations the shear strain cannot be meaningfully applied to the procedures used in this study. Consequently shear ratio is

used. The curve 4.1c can then be replotted in these new terms to yield 4.1d. This graph describes a property of the material from which the cube is made. The graph is unambiguous: for each applied stress there corresponds one shear ratio, and when the stress is removed the shear ratio immediately returns to zero. The rate at which the material deforms does not effect the final magnitude of deformation, nor does it matter whether the force is increasing or decreasing. Any material for which such an unambiguous graph can be drawn is said to be elastic.

Another term can be gleaned from this analysis. In 4.1d notice that at each value of ρ the ratio σ over ρ is the same. This is simply another way of saying that the curve is a straight line with slope σ/ρ . This slope then conveys all the information contained in the graph and has been given a name, the elastic shear modulus or G . A material with a linear σ over ρ curve is said to be "Hookean". A material may be elastic but not have a linear stress-shear ratio curve; for example the curve of 4.1e is unambiguous yet not linear. In this case no single value of G will define the curve, G must be computed for each point.

Molecular Basis For Elasticity

The molecular basis for elasticity in "soft" materials has been the subject of much study. The result of this study is the theory of rubber elasticity. An introduction to the theory may be found in Aklonis, MacKnight and Shen

(1967); or a more thorough treatment in Treloar (1975) or Ferry (1965). The precise thermodynamic and statistical arguments of the theory will not be reviewed here as no use will be made of them per se in this study. The general assumptions and conclusions of the theory are used, however, and will be briefly reviewed here.

The theory of rubber elasticity was first proposed to account for the physical properties of natural rubber. It has subsequently been found to account for the properties of nearly all low modulus (E or G 10^2 to 10^6 N/m) elastic solids; but natural rubber still serves as the best example for an explanation of the theory.

When first collected from the rubber tree, rubber latex is a viscous liquid. In order to become "rubber" it must be vulcanized. This involves mixing the latex with sulphur and heating. Studies of the vulcanization process reveal that before treatment the latex is formed from long, independent polyisobutylene chains, and that during treatment with sulphur these chains are crosslinked to each other by disulfide bridges. Only when these crosslinks are present is the material elastic.

These facts are explained in the following manner: Before crosslinking, the polymer chains of the latex are free to twist and contort at random and thermal agitation (brownian motion) ensures that the chains will be in constant motion. The shape of any one chain at any given moment is thus a random event. The statistics of probability can be applied to the entire group of chains

forming the latex to ascertain the most probable configuration of the chains. If a stress is applied to the latex these kinetically free chains will respond by sliding past each other, each maintaining a random configuration and the material will flow.

Now take an unstressed latex fluid and crosslink the polymer chains. If crosslinks are sufficiently far apart each chain will still be able to assume a random configuration. If, however, a stress is applied to the crosslinked network of chains, this situation is changed. The chains are no longer free to slide past each other. As a consequence, when the material is stretched, the ends of the chains are on the average pulled farther apart in the direction of stretch (see Fig. 4.2). Since random motion yields a certain average end to end distance, and this distance is disturbed upon stretching, it can be shown that the configurations the chains assume when the material is stretched are less random.

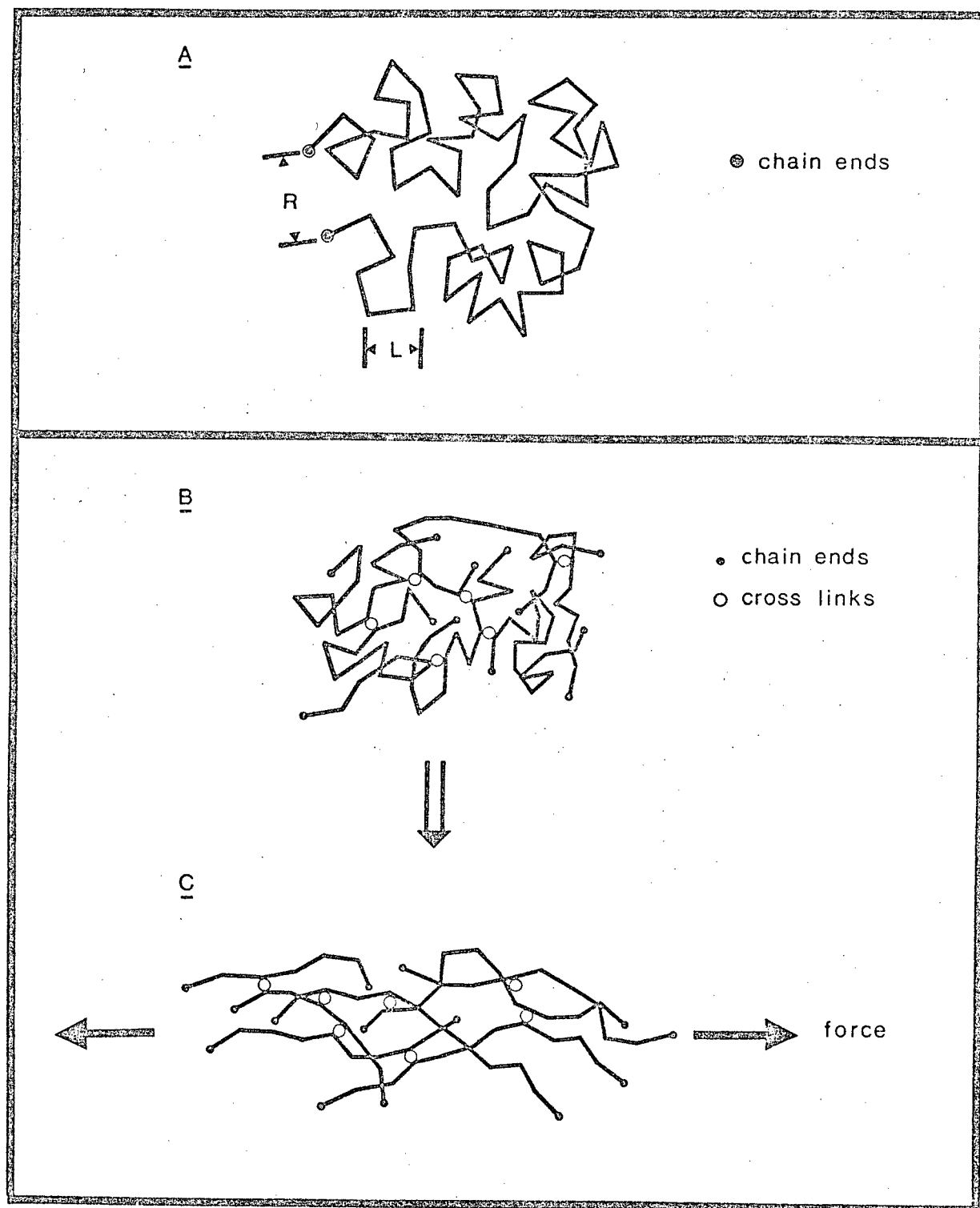
This situation of (undeformed-random) (deformed-less random) can be restated in terms of the conformational entropy of the rubber network. The state of maximum randomness is a state of maximum entropy. A decrease in randomness constitutes a decrease in entropy. Thus by deforming a piece of rubber, one decreases the entropy of the rubber network, a process which under the laws of thermodynamics requires that work be done on the rubber. When the force of deformation is removed the network tends to spontaneously maximize entropy, which is accomplished by

FIGURE 4.2. The molecular basis of rubber elasticity.

A) A long chain consisting of n links, each of length, L , will assume a constantly changing random configuration. The most probable distance between the ends of the chain is $R = (2/3 n L^2)^{1/2}$.

B) A crosslinked, but unstressed network is still randomly arranged. However, when a crosslinked network is stressed (C) R is shifted away from the average value, and the chains are confined to fewer configurations. As a consequence the entropy of the system is decreased.

FIGURE 4.2



returning to the original dimensions and the work of deformation is recovered. Thus a crosslinked network of kinetically free chains is elastic.

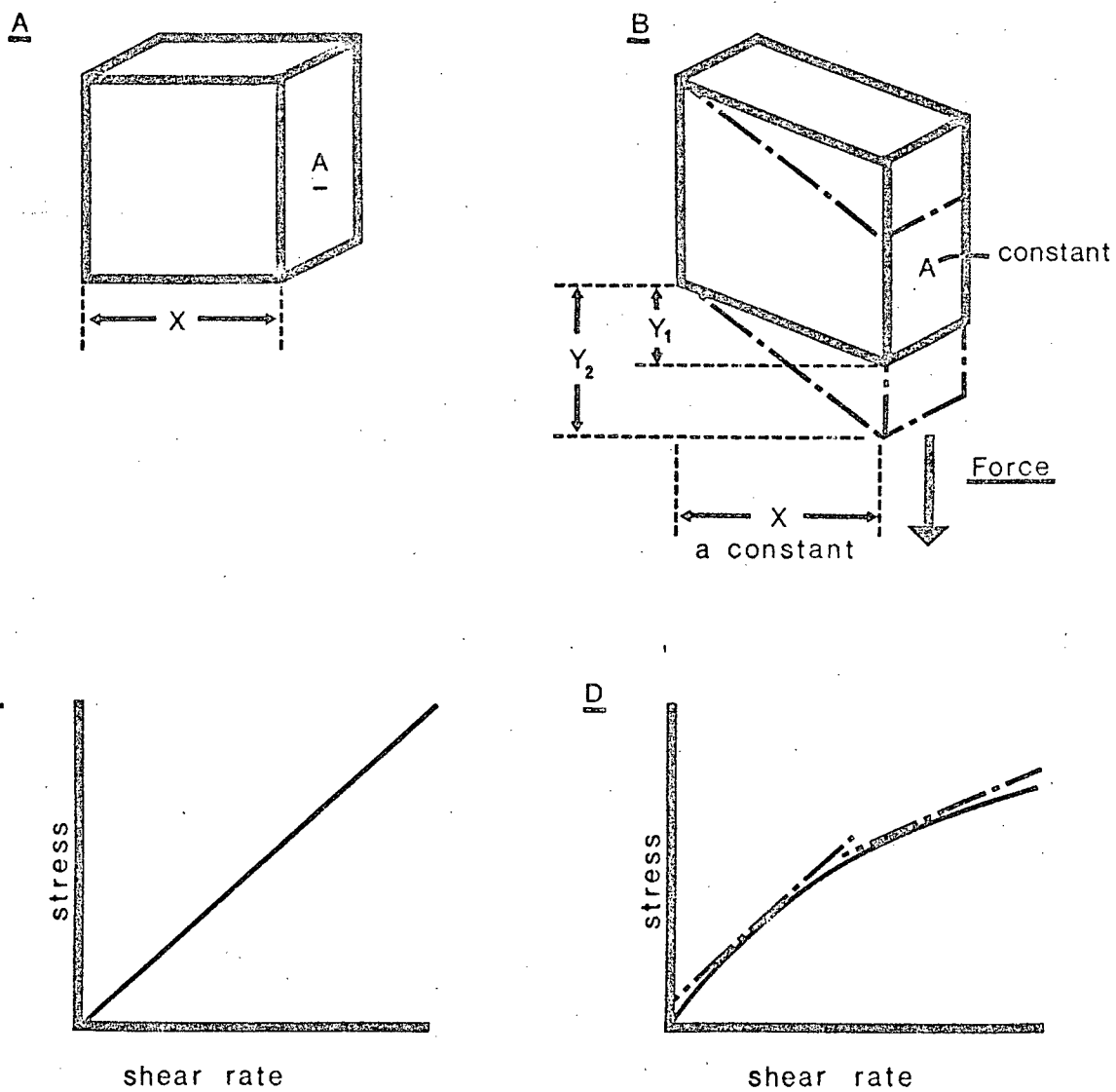
This principle applies to any such network of kinetically free random polymer chains. While it is not logically valid to invert the argument and say that since a soft material is elastic it is formed of a crosslinked network, no other elastic mechanism is known or has been proposed for soft elastic solids. This universal correlation between elasticity of soft materials and crosslinked structure allows one to use the presence of elasticity as strong evidence for the fact that a crosslinked network is present.

Viscosity

The primary characteristic of fluids is that they flow. This concept is quantified in the term viscosity. For the sake of this example imagine that gravity and surface tension do not exist so that a cube of fluid can be formed as shown in Fig. 4.3a. If a stress is applied to the plate as in the previous example the sample will deform (Fig. 4.3b). In this case of a liquid cube, however, the sample will not reach an equilibrium deformation. As long as the stress is applied the sample will deform. If a larger stress is applied the sample will deform faster, but again upon removal of the stress, will not recover its deformation. By applying forces of increasing magnitude a curve can be drawn as in Fig. 4.3c showing the relationship

FIGURE 4.3. The properties of viscous liquids. A given stress applied to a cube of viscous liquid (A) results (B) in a single shear rate (shear ratio/second). The shear rate is proportional to the stress (C). The slope of the stress/shear rate curve is the viscosity. The stress/shear ratio curve need not be linear, as shown in (D), however the viscosity at a point is still the slope of the line at that point.

Figure 4.3



between stress and rate of deformation. (shear ratio) symbolized as $\rho \dot{}$. This graph provides an unambiguous description of a property of the fluid, for each stress there is one shear rate; deformations of the liquid are not recovered.

Again the linear relationship can be characterized by a single number - the slope of the line. This ratio of stress/shear rate is the viscosity. A fluid with a linear stress/shear rate curve is said to be "Newtonian". Fluids showing a non linear stress/shear rate relationship must be characterized by a viscosity at each stress.

Molecular Basis For Viscosity And Flow

The molecular basis of viscosity and flow is at one time both simple and complex. The prime requirement for flow is that the molecules of the material be independent of one another, that is, that a molecule is not energetically restrained to occupying certain fixed positions relative to other molecules. It is on the basis of this criterion that a fluid, in contrast to a solid, can change shape without altering its internal energy. If this requirement were to be strictly met, however, it would require no energy, and hence, no force to deform a fluid and the fluid's viscosity would be zero. That all real fluids have measurable viscosities implies that while individual molecules are not rigidly bound to each other there is some energetic cost that accompanies movement of a molecule from one point to another. The magnitude of the energy cost determines the

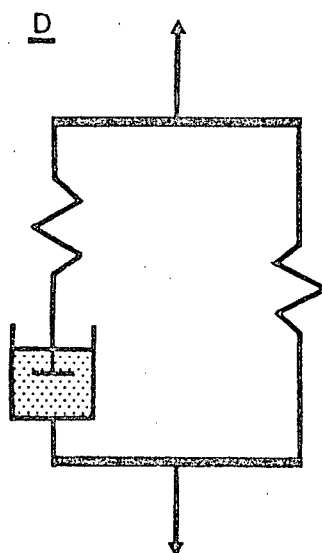
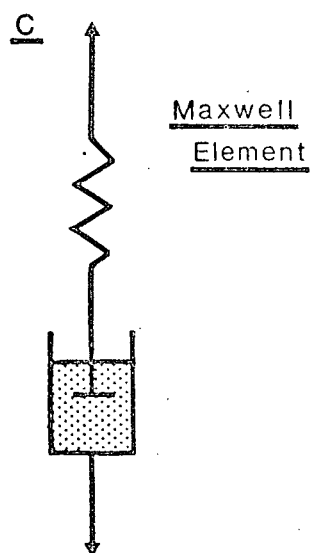
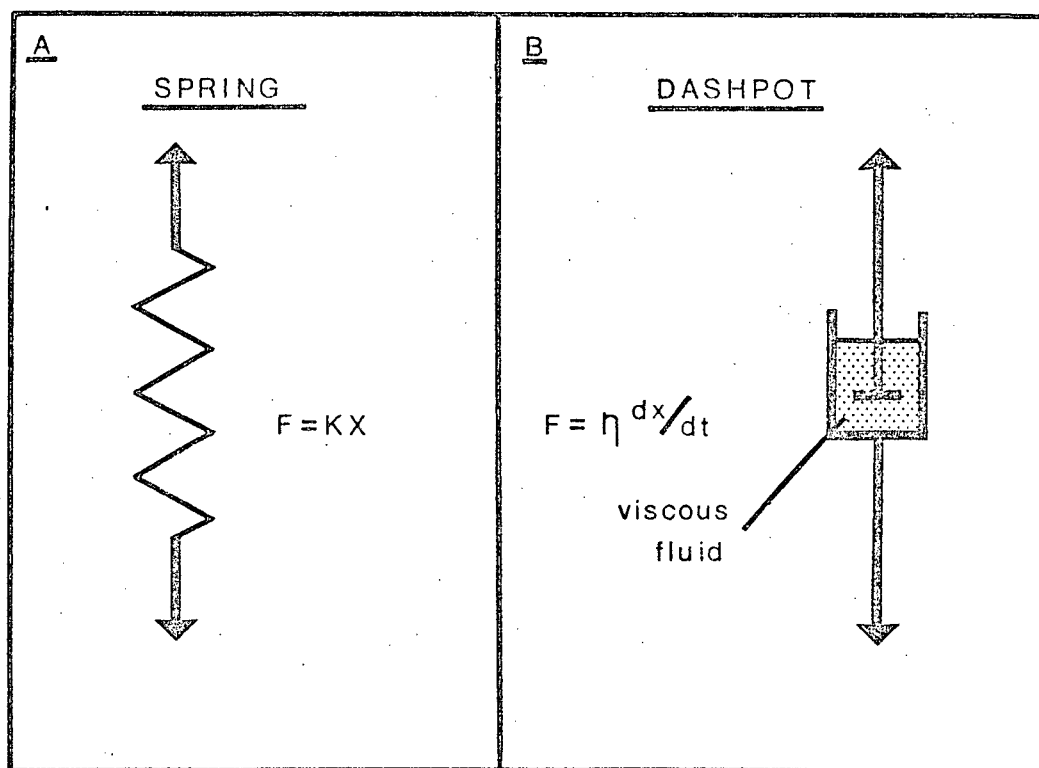
viscosity . Thus it is the hydrogen bonding between molecules which accounts for the high viscosity of water relative to a fluid where no hydrogen bonds are formed, such as benzene. It is difficult, however, to apply this simple concept in any precise fashion to macromolecular fluids. It is usually assumed that flow in these fluids involves the constant breaking and reforming of weak bonds (individual hydrogen bonds, hydrophobic interactions, etc) as molecules move past each other.

Viscoelastic Materials

No biological material is purely an elastic solid or a viscous fluid. As a class , viscoelastic biomaterials show the entire spectrum between solids that will flow slightly if given enough time (bone) to liquids which under proper circumstances may recover from deformation (synovial fluid). In order to describe the properties of these materials a number of standard techniques have been devised. In general the objective of these techniques is to separately quantify the viscous and elastic contributions to the overall properties of the material. In light of this objective it is useful to describe these tests with the aid of some simple mathematical models of viscoelastic materials. These models are constructed from two hypothetical structures: an ideal spring, and an ideal dashpot as shown in Figure 4.4. The spring is used as a model for a material that is purely elastic - the more you deform the spring the harder it pulls back. Thus the equation for the force/deformation curve for

FIGURE 4.4. Springs and dashpots. A spring (A) can be used to model an ideal solid where force, F , is proportional to the displacement, X . A dashpot (B) is used to model an ideal viscous fluid where force is proportional to the rate of displacement. Springs and dashpots can be combined to model viscoelastic materials. A "Maxwell Element" models an uncrosslinked viscoelastic material. A "Maxwell Element" in parallel with a spring (D) models a crosslinked viscoelastic material.

Figure 4.4



a spring is:

$$f=kx$$

where f is force, x is deformation, and k is a constant proportional to the elastic modulus. The dashpot, a piston immersed in a viscous liquid, is used to model purely viscous materials. It follows from the definition of viscosity that the force required to deform a dashpot depends on how fast the dashpot is deformed. Thus

$$f= n \left(\frac{dx}{dt} \right)$$

where n is the viscosity of the fluid in the dashpot. Springs and dashpots can be combined in various configurations to predict the behaviour of viscoelastic materials under various testing conditions.

Stress-shear Ratio Tests

These tests are conducted essentially as depicted in Fig. 4.1a and b. A sample, suitably held, is subjected to an increasing deformation and the force measured at each shear ratio. The deformation may be continued until the material fails ; or at some point the deformation can be reversed and force measured as the sample is returned to its original dimensions. The rate at which the sample is deformed may be varied. Examples of the behavior of viscoelastic materials in this test are shown in Fig. 4.5a,

b, and c. Figures 4.5b and c illustrate the property known as hysteresis, the loss of energy accompanying the cyclic loading of a sample. Hysteresis is a result of the viscous nature of a viscoelastic material.

Stress-relaxation Tests

If a material is deformed to a given shear ratio and the force required to maintain this shear ratio measured as a function of time four general types of curves could be found as shown in Fig. 4.6. If the material is a pure elastic solid (modelled by a spring), by definition the force will not vary with time. If the material is a pure viscous liquid there will be no force since the rate of deformation is zero. If the material is viscoelastic it will show a stress relaxation curve intermediate between the solid and liquid curves. Again, viscoelastic materials may be modelled by springs and dashpots either in series or parallel. A spring and dashpot in series (a "Maxwell Element") will model an uncrosslinked material. An initial force is present due to the stretching of the spring. However, as the fluid in the dashpot flows the force follows an exponential decay to zero force. The time needed to decay to $1/e$ (37%) of the initial force is known as the relaxation time, a measure of the ratio of viscosity to elasticity. A Maxwell Element in parallel with a spring models a crosslinked viscoelastic material. Here, the force decays exponentially with time to a value above zero.

FIGURE 4.5. Properties of viscoelastic materials.

A) A stress/shear ratio plot for a hypothetical viscoelastic material. Each curve represents one sample tested to the point of failure (o). Note that the material is sensitive to the rate of shear; the stiffness increasing as the shear rate increases.

B and C) Energy is lost due to hysteresis in the deformation of viscoelastic materials. The energy required to deform the material is represented by the vertically hatched area (force x distance = energy). The energy not recovered when the sample is unloaded is represented by the horizontally hatched area. Hysteresis equals the energy lost divided by the total energy. In (B), a crosslinked material, the hysteresis is present even though the sample returns to its original dimensions. In (C), an uncrosslinked material, the sample has been permanently deformed by the length, d .

Figure 4.5

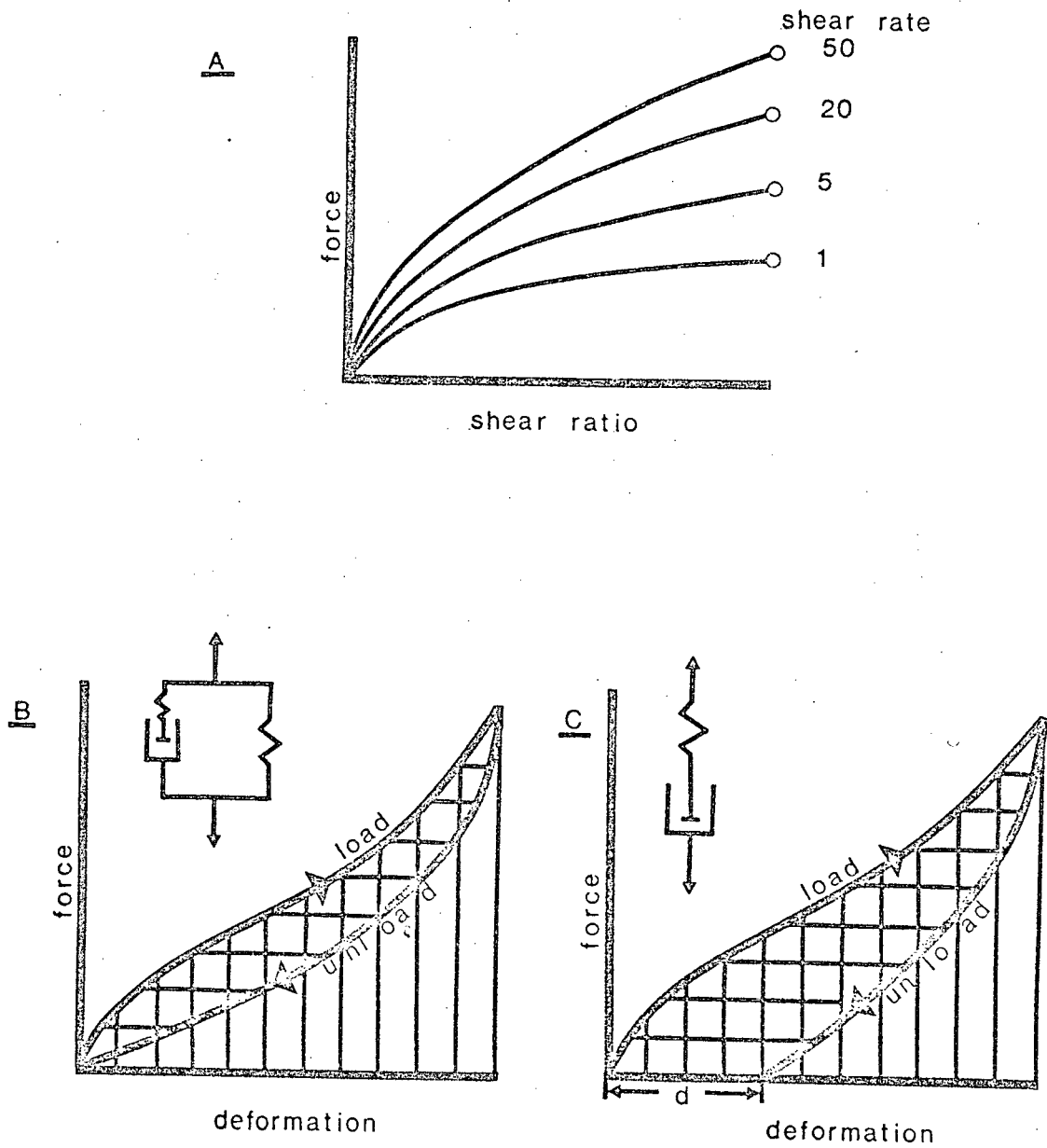
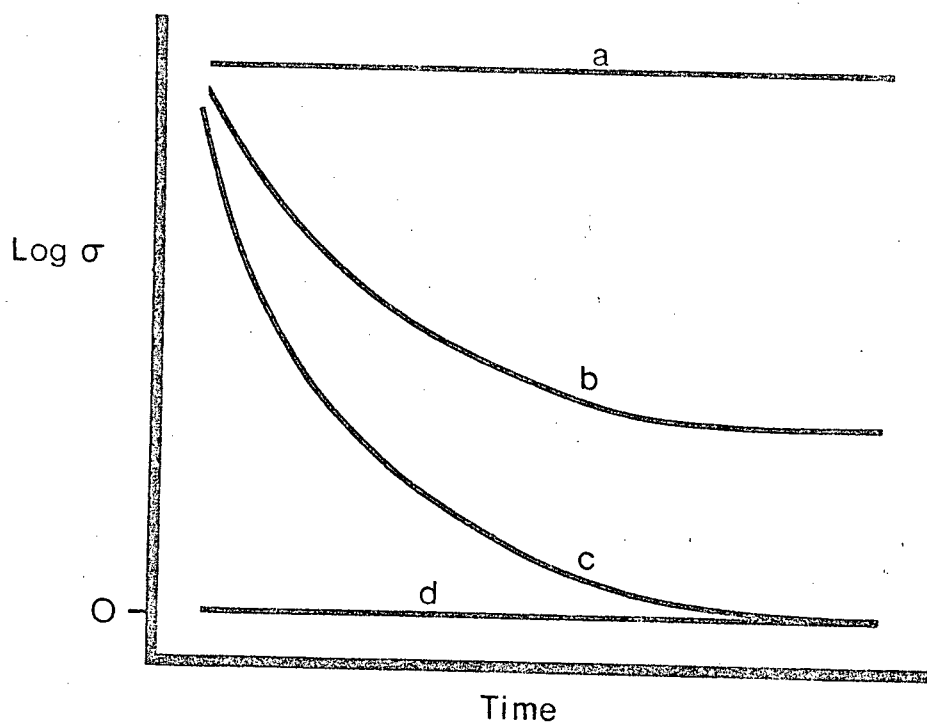


FIGURE 4.6. Representative stress relaxation curves.

- A) An ideal elastic solid (a spring) does not relax.
- B). A croslinked viscoelastic material (Figure 4.4, model D) relaxes to an equilibrium modulus.
- C) An uncrosslinked viscoelastic material (Figure 4.4, model C).
- D) An ideal viscous liquid.

Figure 4.6

Representative Stress Relaxation Curves



Dynamic Testing

As explained above, for an elastic solid force is proportional to the amount of deformation, while for a viscous fluid force is proportional to the rate of deformation. This fact is utilized in dynamic tests to separate the contribution of elasticity and viscosity to the overall properties of a material. What is required to achieve this end is a regimen of deformation where the extent and rate of deformation are separated in time. This is accomplished by sinusoidally deforming the material such that the deformation

$$x = \sin \omega t$$

Since for a spring $f = kx$, the force on the spring due to the sinusoidal deformation will be

$$f = k \sin \omega t,$$

and the force will be in phase with the deformation. For a purely viscous material $f = n \, dx/dt$. Therefore

$$f = n \, d(\sin \omega t)/dt = n \cos \omega t,$$

so that force for a viscous material will lead deformation by 90 degrees (see Figure 4.7). A viscoelastic material will show a phase shift intermediate between a purely viscous or purely elastic material, ie. somewhere between 90 and 0

Figure 4.7: Characteristics of sinusoidal deformations.

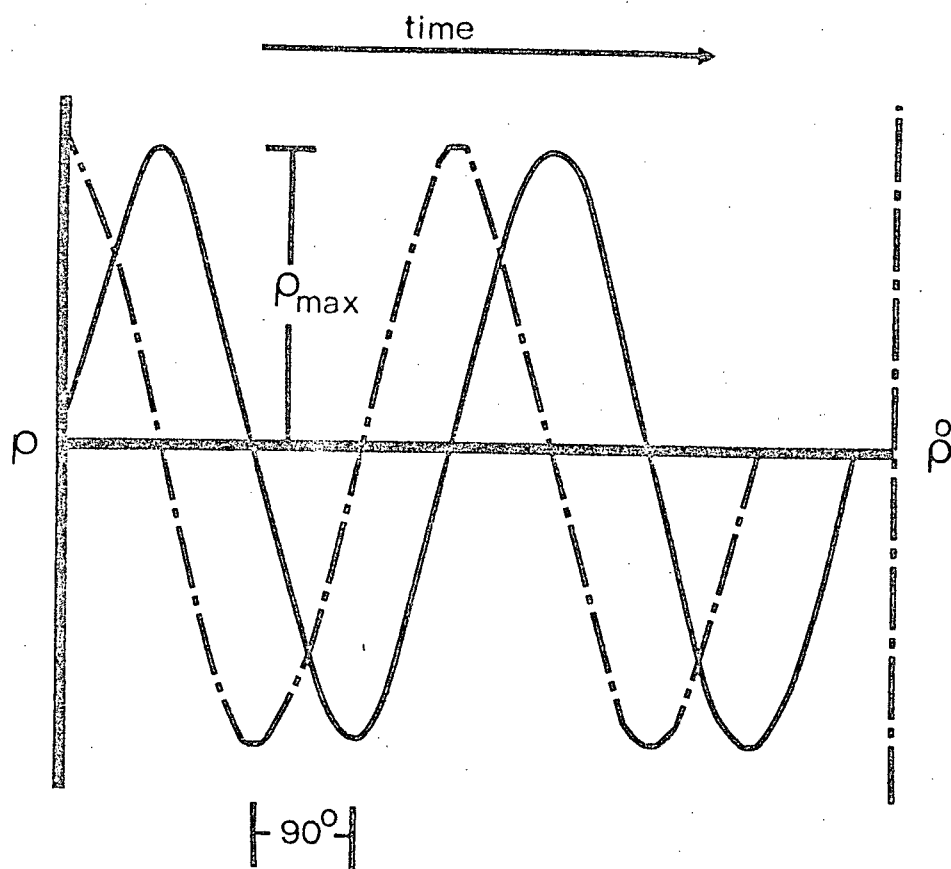
$\rho = \rho_{\max} \sin(\omega t)$, therefore

$\sigma = \sigma_{\max} \sin(\omega t)$ for an elastic solid

$\sigma = \sigma_{\max} \frac{d \sin(\omega t)}{dt} = \sigma_{\max} \cos(\omega t)$ for a viscous liquid

$\cos(\omega t)$ leads $\sin(\omega t)$ by 90°

Figure 4.7



degrees. Thus by measuring the phase shift, δ , between deformation and force a measure of the relative values of elasticity and viscosity can be found. At the same time the ratio of the force amplitude divided by the displacement amplitude will yield a stiffness, or modulus. This overall modulus (the complex modulus) is denoted G^* . The elastic contribution to $G^* \cos \delta$ $G^* = G'$ is called the storage modulus, as it is a measure of the energy stored in each cycle. Obviously $G' = G^*$ when $\delta = 0^\circ$ and $G' = 0$ when $\delta = 90^\circ$. The viscous contribution is $\sin \delta$ $G^* = G''$ or the loss modulus. The ratio of G'' over G' is $\tan \delta$, a measure (at low values) that can be related to hysteresis.

These three techniques form the basis for this study of the physical properties of A. columbianus pedal mucus. The knowledge gained from the tests described here can be used to 1) predict the behaviour of a material in a given situation, and 2) provide some clue to the macromolecular structure of the material.

Testing Apparatus And Procedures

Two types of testing machines were constructed to perform the tests described above. Both machines were designed to test thin layers of mucus in shear, under conditions as closely as possible approximating those under a moving slug.

Dynamic Testing Apparatus

A forced oscillation dynamic testing apparatus was

constructed as shown in Figure 4.8 and 4.9. In this machine the sample is held between two parallel glass plates, the thickness of the sample being measured and set by a micrometer. The plates were aligned daily while being observed with a dissecting microscope. Typical sample thicknesses were 50um to 150um. Sample areas were measured visually by estimating the proportion of the 1 cm² glass plate occupied by the sample. One glass plate is coupled to an electromagnetic vibrator and is the instrument for deforming the sample. The actual displacement of this glass plate was measured by a transducer connected to the vibrator shaft. This displacement transducer was calibrated by deflecting the measuring beam with a micrometer. Within the range of displacements used in this study, the transducer output was linear with displacement. Typical displacements for dynamic tests were 20 um to 50 um. Displacements up to approximately 400 um could be used for stress/shear ratio tests .

The second glass plate is supported by a parallel beam transducer which senses the force exerted on a sample during a test. The force transducer was calibrated by hanging accurately known weights from the center of the glass plate. Forces as small as approximately 10 dynes could be measured accurately. The unloaded resonance of the force transducer is approximately 400 hz.

During a dynamic test the vibrator was powered by a sinusoidal signal generated by the transfer function analyzer. The amplified signals from the force and

FIGURE 4.8. A schematic diagram of the forced oscillation dynamic testing apparatus used to examine the physical properties of pedal mucus at low shear ratios. S= sample; F= force transducer; D= displacement transducer; A= matched carrier amplifiers (SE Laboratories type 4300). The phase analyzer is an SE Laboratories type SM 272DP transfer function analyzer. The vibrator is a Ling Dynamic Systems model 200.

Figure 4.8

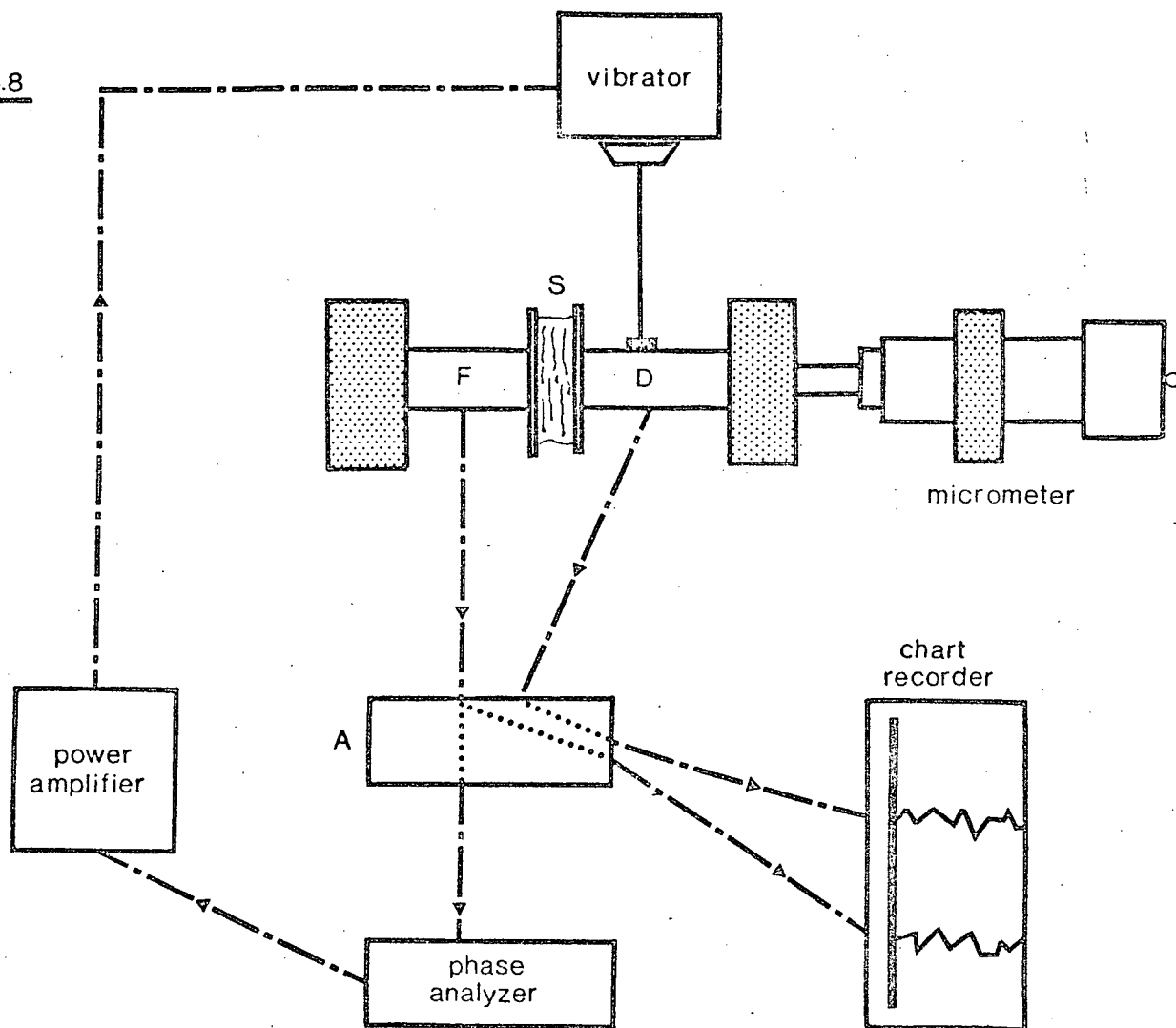
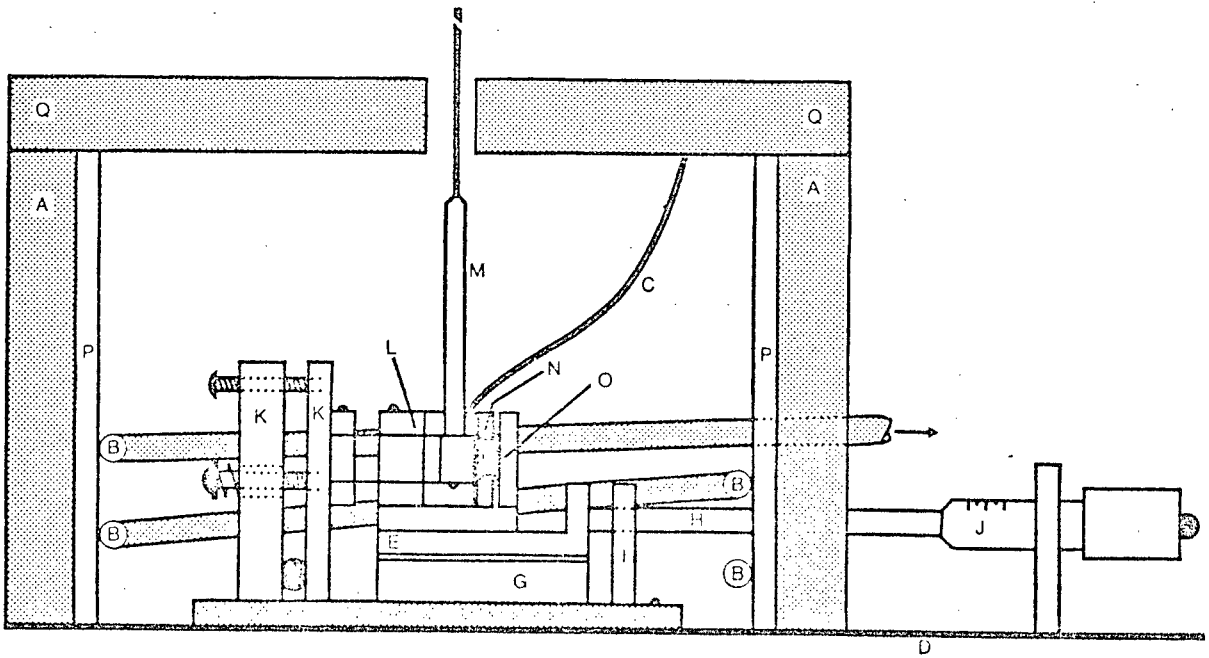
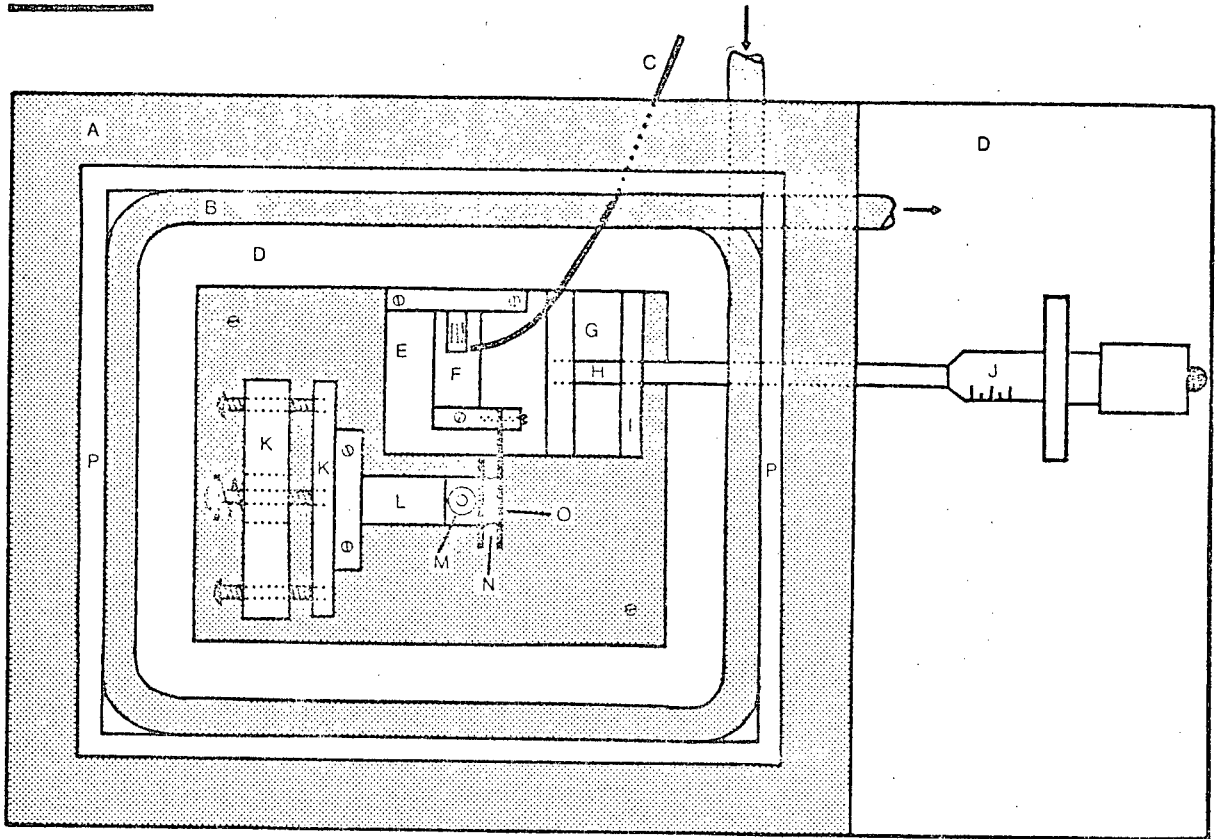


FIGURE 4.9. A construction drawing of the dynamic testing apparatus. A= styrofoam insulation. B= coiled copper tubing carrying water from a controlled temperature bath. C= shielded cable from the force transducer. D= base plate. E= sliding force transducer assembly. F= stainless steel beam (0.008" thick) with mounted semiconductor strain gauges (BLH type SPB3-20-35). G= glass sliding surface. H= rod connecting the force transducer to the micrometer. I= guide bearing for the micrometer rod. J= micrometer, fixed to the base plate. K= the alignment system for the displacement apparatus. L= stainless steel beam (0.002" thick) supporting the displacement glass plate. M= rod connecting the displacement apparatus to the displacement transducer and vibrator. N= mucus sample sandwiched between the force and displacement glass plates. O= the force glass plate p= Plexiglas container. Q= styrofoam insulating cover with a gap for the displacement rod.

FIGURE 4.9



displacement transducers were in turn compared to this reference signal. For each signal the analyzer calculates the phase shift from the reference sine wave and the amplitude of the transducer signal. From these data the phase shift of the force signal relative to the displacement signal could be calculated to yield a value for δ . The ratio of amplitudes is a measure of G^* . Samples were tested at frequencies ranging from 0.2 hz to 100 hz. Above 100 hz resonances within the frame supporting the vibrator cause spurious and erratic readings. Below 0.2 hz drift in the semiconductor strain gauges of the force transducer does not allow for accurate measurements.

Stress-shear ratio tests were performed by powering the vibrator with a triangular wave. Amplified signals from the force and displacement transducer are then supplied to a two channel chart recorder. Both the frequency and amplitude of the displacement could be varied within the limits described above.

The testing apparatus was enclosed in an insulated, temperature controlled chamber that could either be used as a bath to immerse the sample in a test solution or a closed chamber to maintain 100% relative humidity during a test. All tests were conducted at 22-23 °C with temperature variations during the course of a test less than 0.1 °C.

The usefulness of this testing apparatus was limited in two respects. 1. The semiconductor strain gauges used in the force transducer are exceedingly temperature sensitive. While this posed no problem for tests of short duration such

as dynamic or stress/shear ratio tests, tests requiring long term stability (such as stress relaxation tests) could not be performed. 2. The necessity of maintaining the glass plates parallel and a set distance apart limited the shear ratios that could practically be obtained.

In order to overcome these limitations the second testing apparatus was constructed .

The Cone And Plate Apparatus

The cone and plate machine used for measuring the physical properties of slime at large shear ratios and shear rates and for stress relaxation tests is shown in Figure 4.10 . In this machine the sample is held between an aluminum plate and a small angle Plexiglas cone. One revolution of the cone produced a uniform shear ratio in the sample of 249. The geometrical basis for uniform strain in a cone and plate configuration is explained in Figure 4.10. The diameter of the sandwiched sample was measured with vernier calipers and the area calculated .

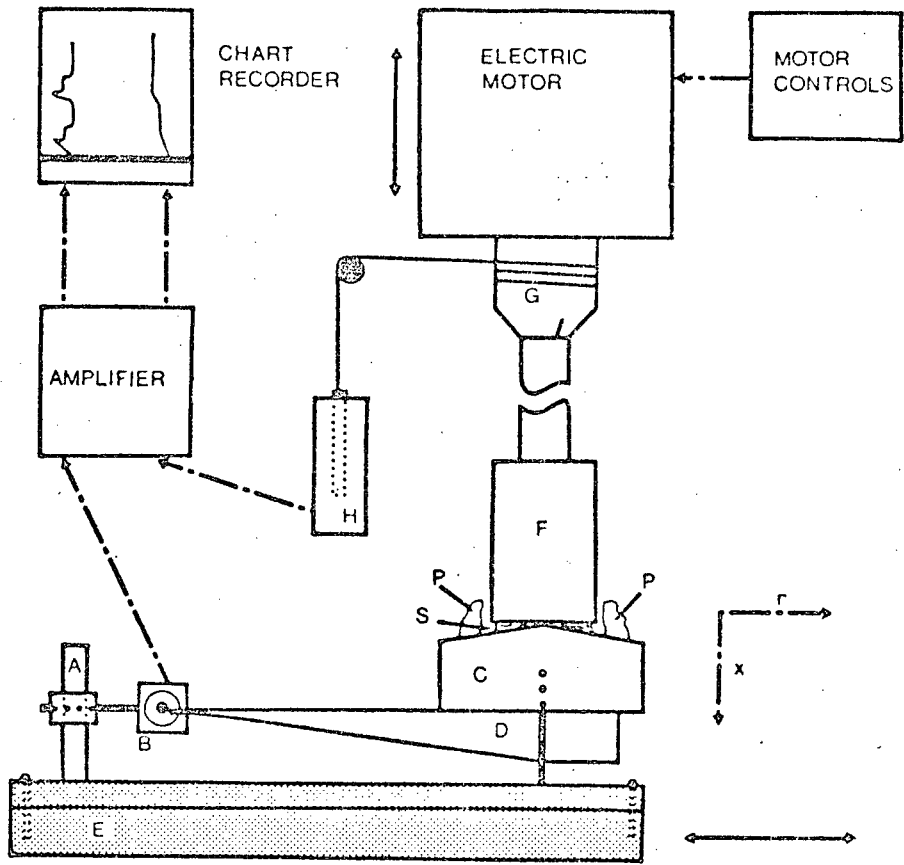
The rotation of the plate was converted to a linear measure by a windlass which supports the core of a linearly variable differential transformer (LVDT). The amplified signal from this transformer was recorded on one channel of a two channel chart recorder. The LVDT was calibrated by inserting the core with a micrometer. Rotations could be measured to approximately ± 2 degrees.

The cone is supported by a torsion bar. The twisting of this bar , a measure of force, results in the deflection

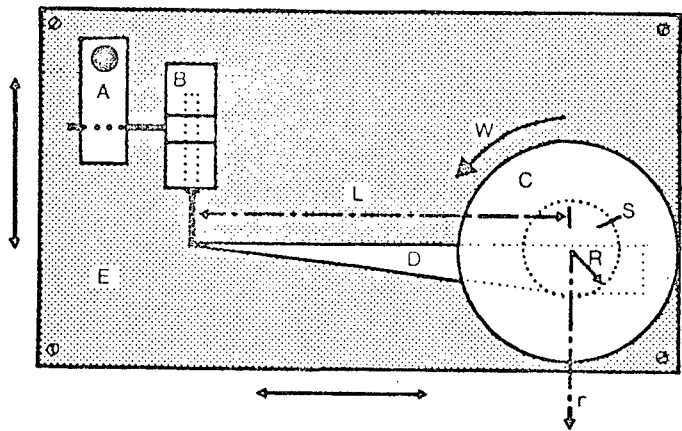
FIGURE 4.10. A combined schematic and construction drawing of the cone and plate apparatus used to examine the physical properties of pedal mucus at high shear ratios. The distance through which the sample is deformed is a function of r , the radius, and W , the angular velocity and is equal to rW . The thickness of the sample is again a function of the radius and is equal to mr where m is the slope of the cone. The shear ratio thus equals $Wr/mr = W/m$ and is independent of the sample radius. The effective sample radius is the radius of a cylinder coaxial with the cone that contains one half of the sample volume. It is approximately equal to $0.79 R$. The electric motor is a Cole-Parmer Master Servodyne, and the amplifiers are SE Laboratories type 4300. A= adjustable mounting post for B, a Schaevitz 050 MHR linearly variable differential transformer (LVDT). C= Plexiglas cone supported by an 18 gauge hypodermic needle. D= arm supporting the LVDT core. E= base plate bolted to the horizontally adjustable stage of a milling machine. F= aluminum rod with a polished end acting as the plate. G= chuck attaches the rod to the motor and acts as a capstan. H= Schaevitz 100HR LVDT. L= distance from core to the center of the cone. P= moist paper to slow evaporation rate from the sample. S= sample. The electric motor is mounted on the vertically adjustable post of a milling machine.

FIGURE 4.10

A



B



of a rigid arm which supports the cone of a second LVDT. The signal from this transformer was recorded on the second channel of the chart recorder . The force transducer was calibrated by turning the transducer on its side so that the LVDT is vertical. Accurately known weights were then hung from the core. The force generated during a test was assumed to act at a radius R as explained in Figure 4.10. This radius was then compared to the distance from the center of the cone to the LVDT core to calculate the mechanical advantage and arrive at a final calibration . Forces as small as 100 dynes (acting at a typical effective sample radius of approximately 5 mm) could be accurately measured. The unloaded resonance of the force transducer was approximately 50 cycles per second.

The electric motor rotating the plate provided shear ratios continuously variable above shear rates of 5 per second. The time required to reach full speed was about 20 milliseconds.

All tests were performed at room temperature (21-24 °C) with no provision made to regulate the temperature of the sample and apparatus. Temperature variations during tests were less than 0.5 °C. A moist ring of absorbent paper placed as shown in Figure 4.10 serves to maintain a high relative humidity around the sample and thereby minimize evaporation.

Collection Of Pedal Mucus

Ariolimax columbianus pedal mucus was collected by

allowing the slug to crawl on a glass rod. As the slug attempted to crawl around the rod the rod was rotated forcing the slug to continue crawling lest it lose its footing. In this manner 0.1 to 0.3 ml of pedal mucus could be collected from each slug. This amount was sufficient for any of the tests described above to be performed on a single sample from one individual. The collection procedure lasted approximately one minute. Samples were placed in the testing apparatus immediately after collection. No attempt has been made to determine the precise origin of the pedal slime collected, though it is probable that the preponderance was produced by the pedal gland. By dusting the dorsal surface of a slug with chalk prior to pedal mucus collection it was shown that the mucus collected from the foot was not contaminated by mucus from the dorsal epithelium.

It is inevitable that variations be found in the properties of slime from one slug compared to that of another slug, or that slime properties will vary when collected from one slug on different days. For example, in one set of 18 slime samples collected on a single day, the dry weight of the mucus ranged from 2.85% to 4.46% of the wet weight. In attempting to accurately describe the physical properties of slug slime an account of this variability must be made when reporting results. In the case of certain tests variation in the composition of the collected slime appears to cause little variation in the property measured. In these cases information about

individual samples is not lost in looking at an average of all samples, consequently results of all tests are averaged and the confidence limits around the mean are noted. Other tests are affected by compositional variability to a greater extent. For these tests it often happens that the average of all tests does not closely resemble the measured value for any individual test. In this case the results from the individual sample closest to the average will be presented as a "typical" test and the range of values for all tests will be noted.

Physical Properties Of A. Columbianus Mucus At Low Shear Ratios

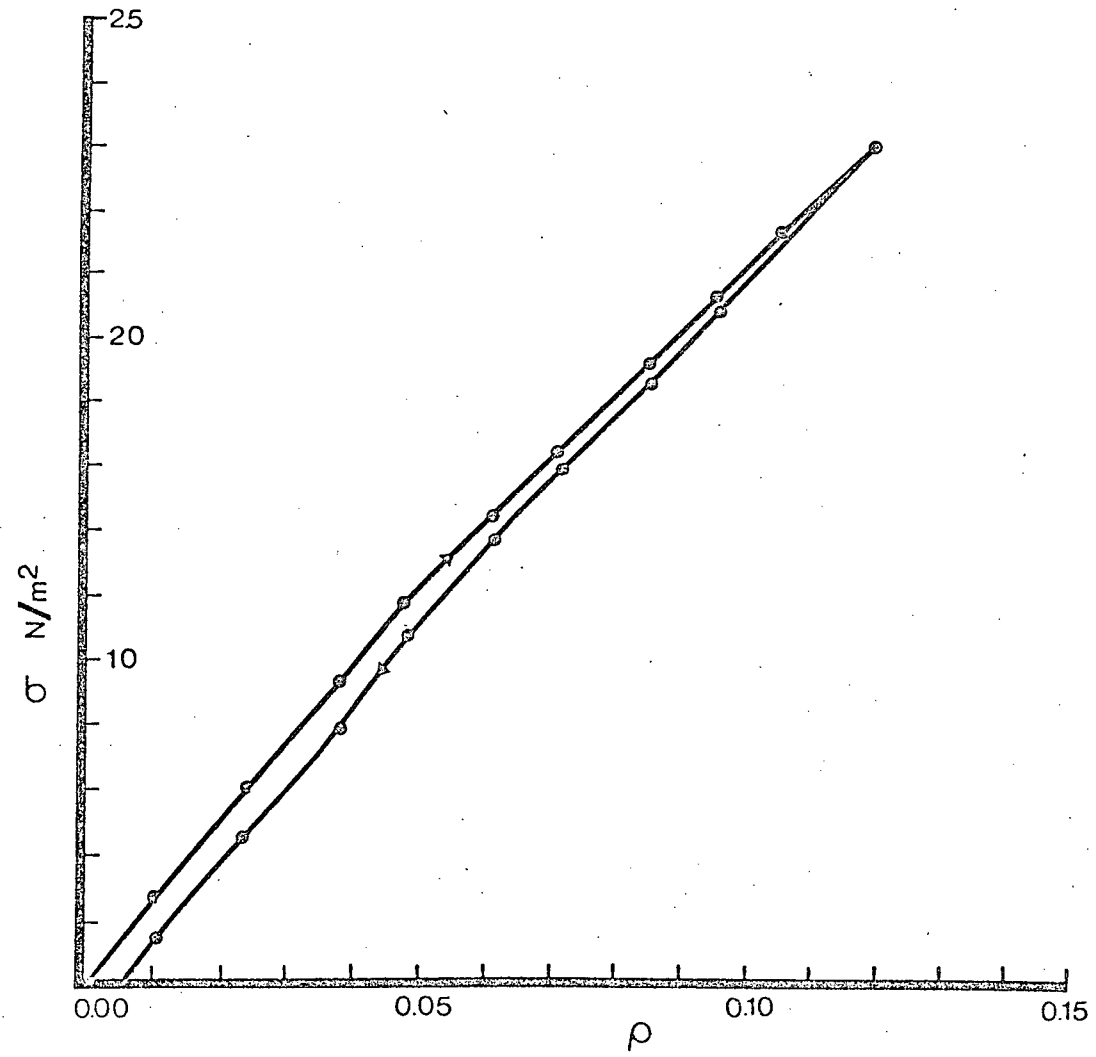
Three sorts of tests were conducted to ascertain the properties of *Ariolimax columbianus* pedal mucus at low shear ratios (ie. less than 5) : 1) Stress/shear ratio tests 2) Stress relaxation tests and 3) Dynamic tests. The results of these tests will be discussed in turn.

Stress-shear Ratio Tests

Figure 4.11 shows a typical stress- shear ratio curve for mucus deformed in the dynamic testing apparatus to a shear ratio of a few percent at a low shear rate. Under these conditions the slime behaves primarily as an elastic solid. The stress/shear ratio curve is essentially linear with an elastic modulus of approximately 200 N/m². That the mucus is elastic at these shear ratios and shear rates indicates the presence of some network structure within the

FIGURE 4.11. A stress/shear ratio curve for Ariolimax columbianus pedal mucus at a low shear ratio. Shear rate = 0.048/sec. and $G = 210 \text{ N/m}^2$. The hysteresis is 6.9%.

Figure 4.11



slime. The viscous nature of the material shows only in the slight hysteresis.

Figure 4.12 shows a typical stress - shear ratio curve for mucus at larger shear ratio and at a shear rate over ten times that of Figure 4.11. Under these conditions the viscoelastic nature of the material is evident. Upon loading the mucus again shows a G of about 200 N/m^2 but upon unloading it can be seen that a considerable proportion of the energy of deformation has gone to deforming the viscous component of the material, and is non-recoverable. The fact that the material does not return to its original dimensions indicates that whatever network is responsible for the elasticity is either broken down partially or has rearranged in the course of deformation. However, the fact that the mucus does recover some of the deformation shows that some form of elastic network is still present.

Stress Relaxation Tests

Figure 4.13 shows the averaged results from 10 stress relaxation tests carried out in the cone and plate apparatus. These tests were done at a range of shear ratios from 2 to 5. No difference in the time course of relaxation as a function of shear ratio was noted. The relaxation curve cannot be characterized by a single or small number of relaxation times indicating that there are a variety of different relaxation processes occurring in the material. This is typical for viscoelastic biomaterials. Unfortunately this form of test does not give any clues as

FIGURE 4.12 a stress/shear ratio curve for Ariolimax columbianus pedal mucus at a moderate shear ratio. Shear rate = 0.56/sec. and G is approximately 100 N/m^2 . The hysteresis is 44.2%. Note that the sample does not return to its original dimensions.

Figure 4.12

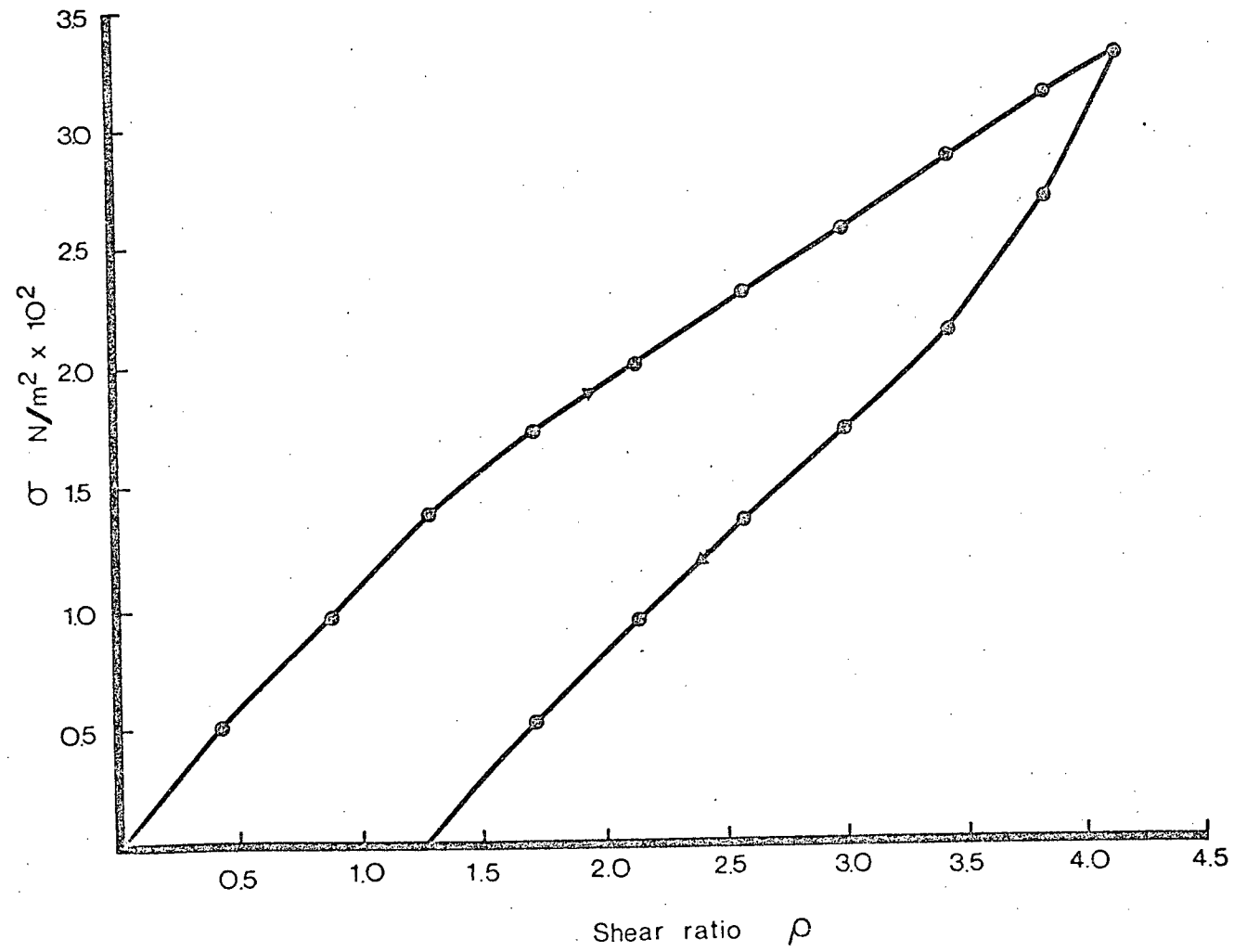
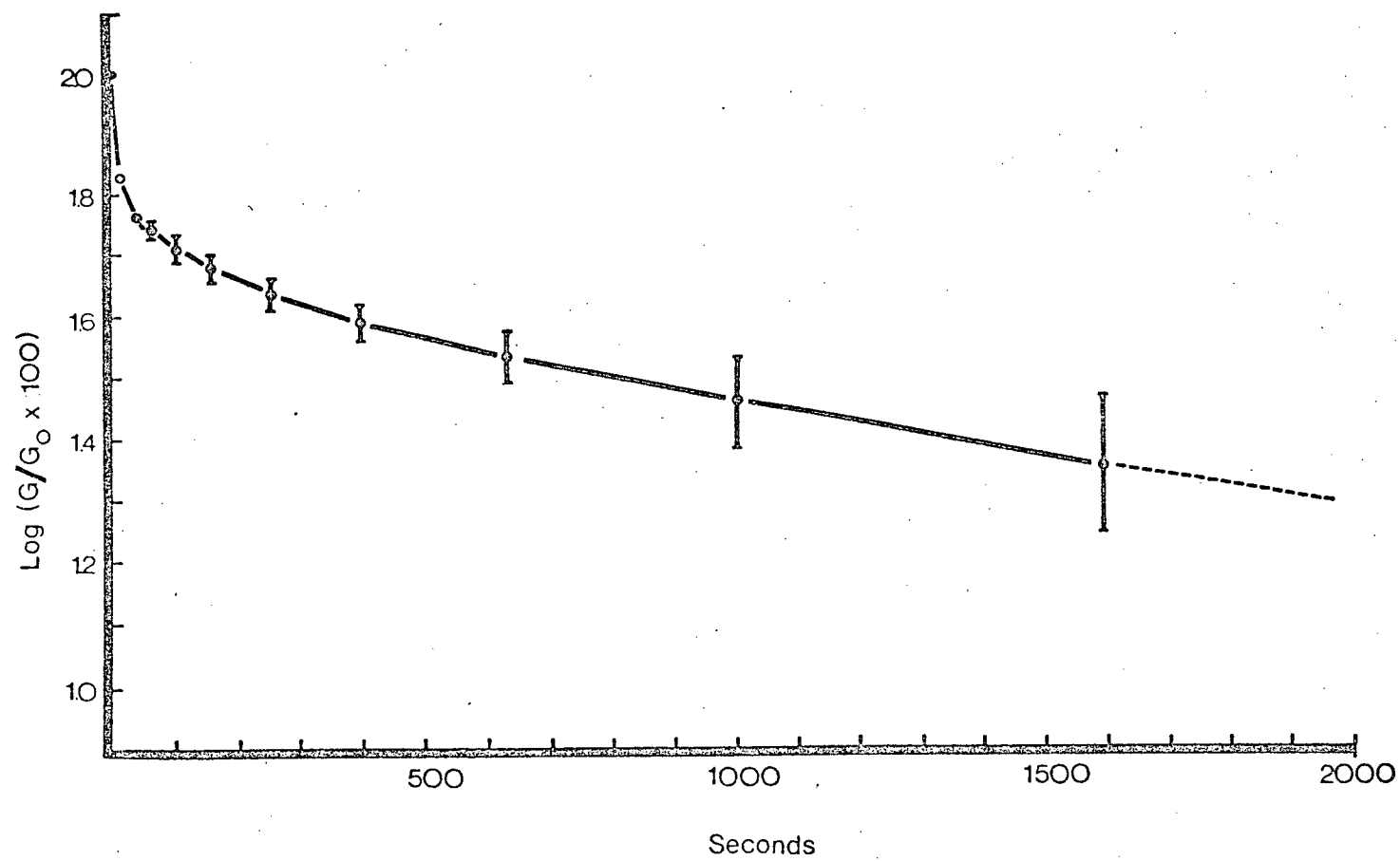


FIGURE 4.13. The stress relaxation characteristics of Ariolimax columbianus pedal mucus. The curve is the average of 10 tests; the bars represent 95% confidence intervals.

Figure 4.13



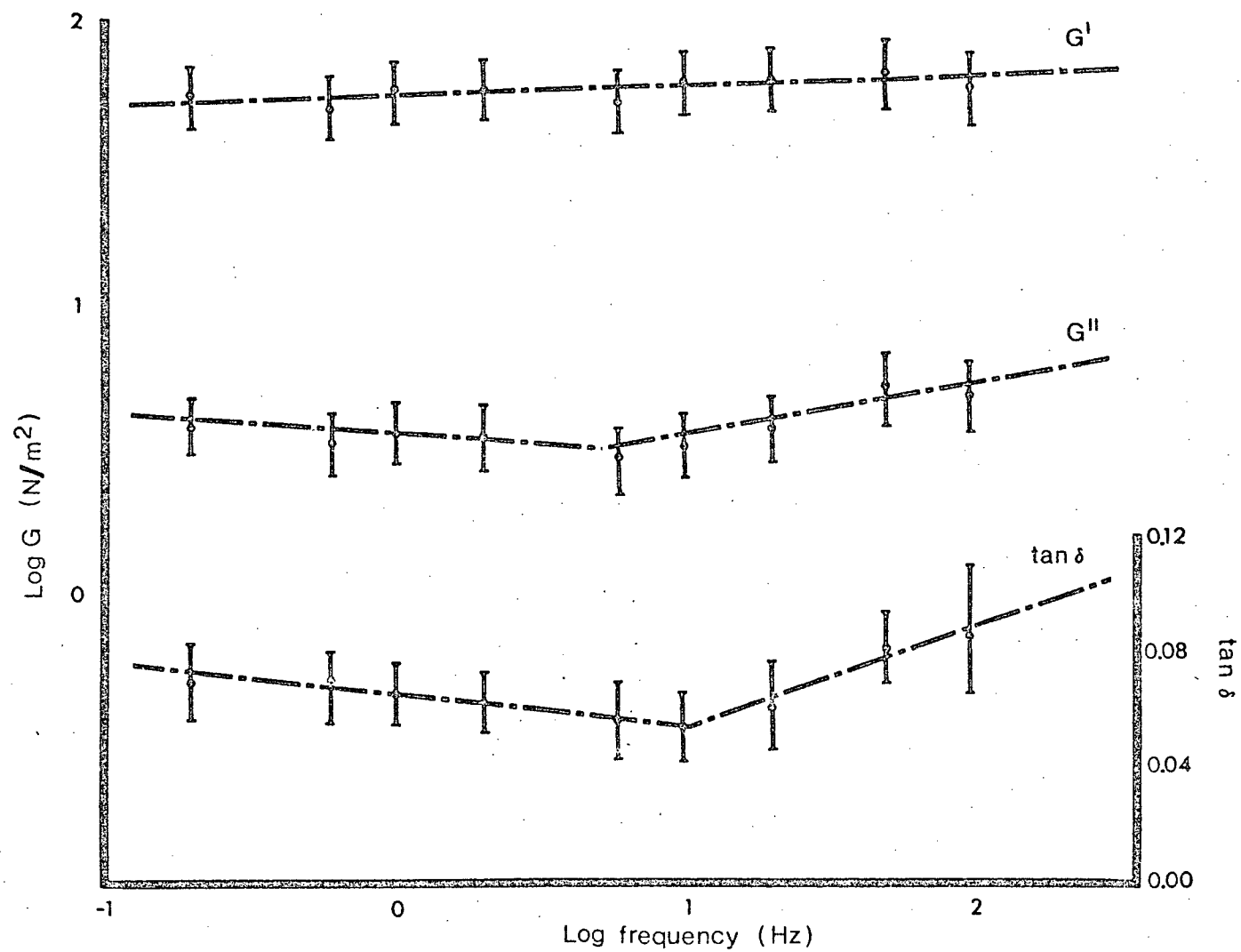
to the nature of these relaxation processes. Within the time course of these experiments (30 minutes) the relaxing slime does not reach an equilibrium stress, nor does the curve give any hint that an equilibrium would be reached if relaxation for greater periods of time could be measured. In this respect, in that it flows, mucus behaves as a fluid. However, it has been shown by stress - shear ratio tests that an elastic network is present during deformation to these shear ratios for at least short time intervals. From these two facts it can be hypothesized that this network, while stable over relatively short periods of time (seconds), is capable of rearrangement over long periods of time (minutes to hours).

Dynamic Tests

The results of the dynamic tests on Ariolimax columbianus pedal mucus are summarized in Figure 4.14. As mentioned earlier these tests were carried out at low extension ratios of about .2 to .5 and frequencies from .2 to 100 Hz. These tests are thus designed to reveal the properties of the elastic network on a time scale of milliseconds to seconds. Under these conditions the material is again shown to behave primarily as an elastic solid: the storage modulus is ten times the loss modulus and the ratio of the two ($\tan \delta$) does not vary significantly over the range of frequencies tested. This confirms that the network of the mucus is kinetically free (and thereby elastic) on a time scale of 10 milliseconds. This is hardly

FIGURE 4.14. Dynamic test results. The curves are averages from 6 samples tested in air at 100% relative humidity. The bars are 95% confidence intervals.

Figure 4.14



surprising since in a network as diffuse as mucus there is virtually nothing present to inhibit the freedom of network chains. It is however further evidence supporting the hypothesis that the elastic network of the pedal mucus of Ariolimax columbianus can be treated realistically as a network conforming to the theory of rubber elasticity.

In summary, the tests on Ariolimax columbianus pedal mucus at low shear ratios indicate that the material, while viscoelastic, is primarily a solid formed of a crosslinked network, the crosslinks being stable on a time scale of milliseconds to seconds, but unstable at longer periods of time.

Physical Properties Of A. Columbianus Pedal Mucus At High Shear Ratios

While it is useful to know the properties of mucus at low shear ratios, the applicability of this knowledge to the problem of slug locomotion is restricted. For a slug with a step length of one millimeter and a mucus layer thickness of 10 micrometers, the pedal mucus will be exposed to a shear ratio on the order of 100 rather than the 0.1 to 4.0 typical of the tests described above. In order to investigate the properties of A. columbianus pedal mucus at higher shear ratios a number of tests were carried out using the cone and plate apparatus.

Stress-shear Ratio Tests

The results of shearing pedal mucus to higher shear

ratios are shown in Figures 4.15 and 4.16. It is evident even at a first glance that these properties are quite different from those at low shear ratios. These differences are best explained by following the time course of a test as shown in Figure 4.15. The test is initiated when the motor rotating the plate is switched on. As the plate rotates through the initial few degrees the mucus sample is deformed as in the stress-shear ratio tests described earlier. Stress rises roughly linearly with shear ratio with a modulus of about 100-200 N/m². However, at a shear ratio of about 5-6, the mucus abruptly yields and the stress falls. Due to the design of the apparatus the shear ratio at which the mucus yields can only be determined to an accuracy of about plus or minus 1.4 rho. Within the limits of accuracy of the machine the breaking shear ratio does not vary with the shear rate at which the sample is deformed. In contrast, the stress at which the sample yields ($\sigma_{sub y}$) is dependent on the shear rate; the higher the shear rate the larger the yield stress. A plot of yield stress versus strain rate for a typical sample is shown in 4.16. As the mucus is deformed beyond its yield point a new stress level is reached. This level remains constant with further deformation. As shown in the introduction to this chapter, the maintenance of a constant stress for a constant shear rate is one distinguishing characteristic of a fluid. It would thus appear that as the mucus yields its elastic network structure is broken to the point where the material behaves as a viscous liquid. If this is so, two other facts

FIGURE 4.15. The characteristics of Ariolimax columbianus pedal mucus at high shear ratios. The mucus yields at a shear ratio of approximately 5 to form a viscous liquid, but will "heal" if allowed to rest unstressed.

Figure 4.15

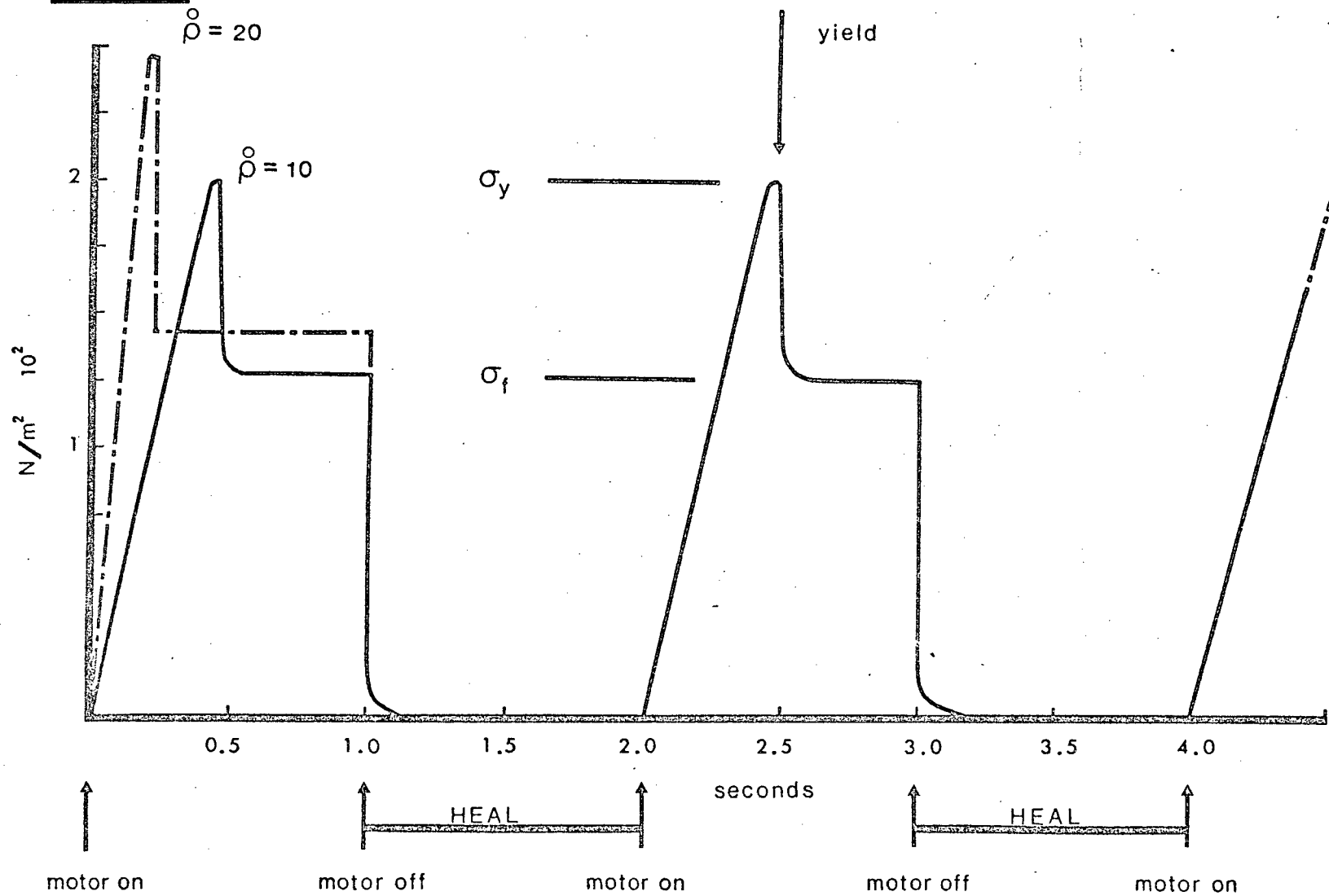


FIGURE 4.16. A representative plot of yield stress and flow stress versus shear rate for Ariolimax columbianus pedal mucus.

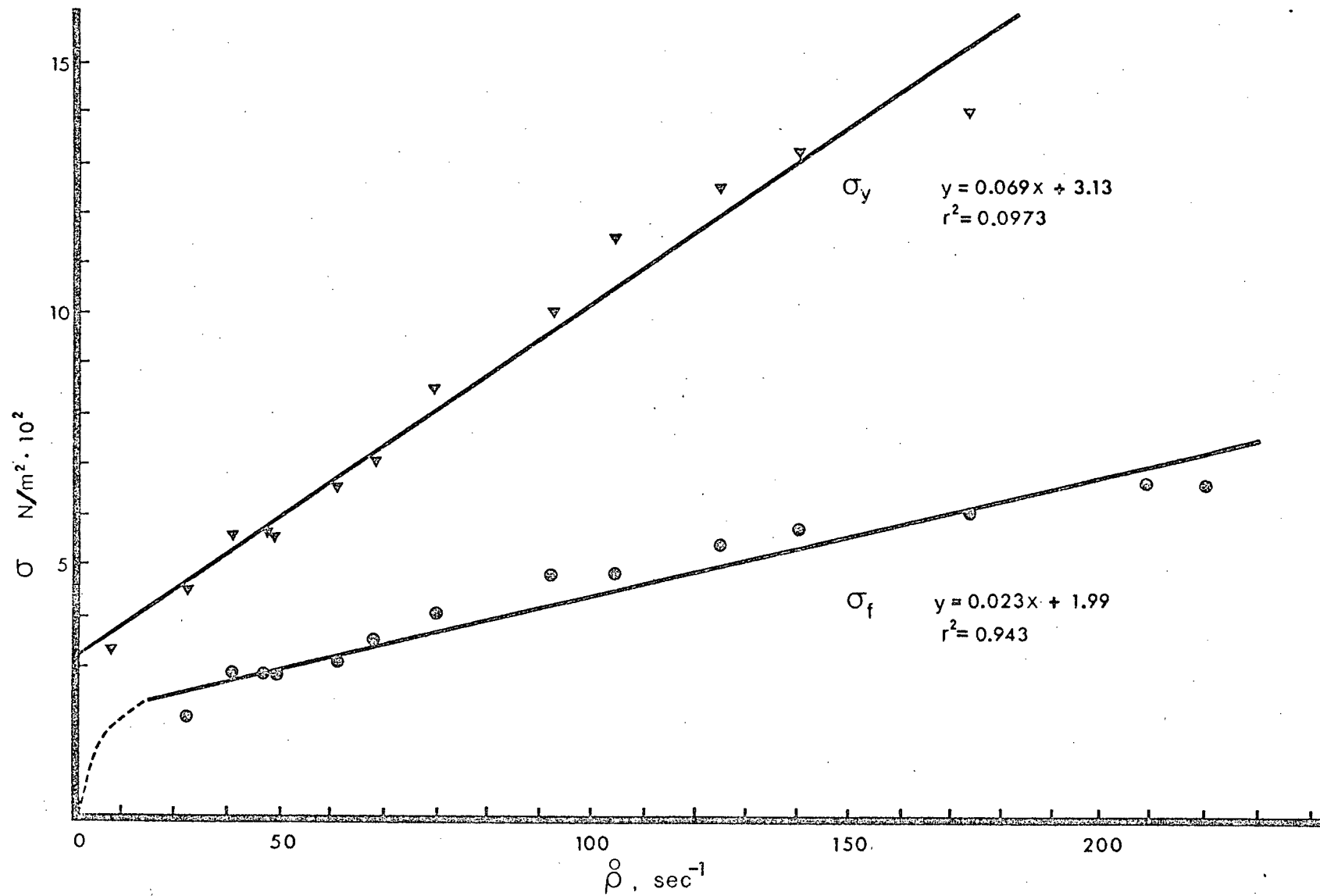
Yield stress range:

$$\begin{array}{ll} \text{low} & y = 0.024x + 0.83 \\ \text{high} & y = 0.117x + 1.92 \end{array}$$

flow stress range

$$\begin{array}{ll} \text{low} & y = 0.010x + 0.070 \\ \text{high} & y = 0.053x + 0.53 \end{array}$$

Figure 4.16



should be consequent: 1) The stress required to deform the liquid should be dependent upon shear rate. This is indeed the case. Samples deformed at higher shear rates show higher post-yield stresses, the flow stress (σ_f). A plot of flow stress versus shear rate for a typical sample is shown in Figure 4.16. The shape of the relationship is linear, showing that mucus, after it has yielded, behaves as a liquid with an incremental viscosity of about 50 poise ie 5,000 times more viscous than water. Note that the line of σ_f does not extrapolate to zero. It is assumed that at shear rates lower than those measured the fluid mucus shows non-Newtonian behaviour as depicted by the dotted line on the graph. 2) When deformation is halted a solid will maintain a positive value of stress; in the case of a liquid the stress will immediately decay to zero. As shown in Figure 4.15 the highly deformed mucus again behaves as a liquid.

Two physical interpretations are consistent with these results. First, the elastic network may break down throughout the entire sample allowing the sample as a whole to behave as a liquid. Second it is possible that only the network in the portion of the sample adjacent to either the cone or plate is destroyed, forming a thin layer of liquid separating the still solid sample from the cone or plate. In this second case it would essentially be the properties of this thin layer that are being tested rather than the sample as a whole. If this is so, and depending on the thickness of the fluid layer, the calculated values of shear

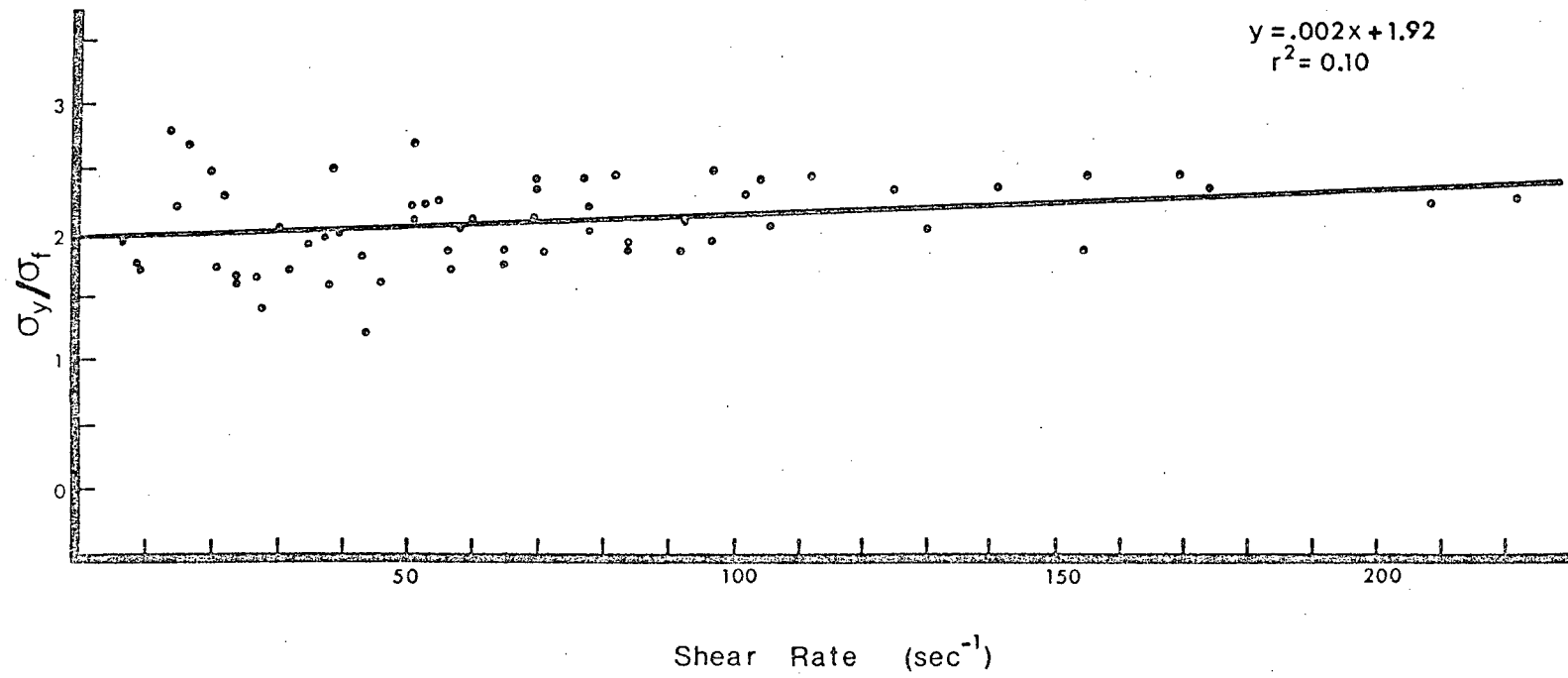
rate may be considerably in error. It is impossible from the tests performed in the course of this study to distinguish which of these two possibilities is the correct one. While it would be interesting to be able to resolve this question, the fact that it has not been resolved does not affect the accuracy of the results of these tests in describing the effective properties of slug pedal mucus.

As shown in Figure 4.16 there is considerable range in the values for breaking stress and flow stress in the samples tested. Though the hypothesis was not tested it seems likely that these variations are due to the normal variation in the concentration of glycoprotein present in the mucus as explained earlier in this chapter. This variation in the magnitude of yield stress and flow stress does not, however, affect the ratio between the two for any one sample nor is the ratio dependent on shear rate. Figure 4.17 shows a plot of the yield stress/flow stress values for all samples tested. This constant value of yield stress /flow stress equal to about 2.0 is important for the locomotion of the animal. It will be shown in Chapter 7 how the ability of the mucus to change from a solid to a liquid is a necessity for adhesive locomotion. The invariant ratio of solid strength to viscosity described here means that the mucus can function effectively within a wide range of hydrations and shear rates.

Do these tests then imply that the mucus beneath a moving slug is in the form of a liquid? One is reminded that as the slug moves the mucus is deformed to a shear

FIGURE 4.17. The ratio of yield stress to flow stress is constant among samples and regardless of shear rate.

Figure 4.17



ratio of about 100.

This question is answered in the course of further testing. Again refer to Figure 4.15. After the rotation of the plate is stopped and the stress has decayed, the sample is allowed to remain unstressed for one second. This period was chosen as equivalent to the period of time that mucus would be beneath the interwave (and therefore not deformed) under a moving slug. At the end of this time the plate is again rotated. It is found that the mucus, rather than showing the characteristics of a liquid, has "healed" and again behaves as a solid. Stress rises linearly with shear ratio. At a shear ratio equal to 5-6 the material again yields and so forth. The record of this second period of deformation is identical to the first. In fact this "yield-heal" cycle can be repeated 20 to 30 times before the mucus begins to show signs of failing to recover its solidity. Thus, the elastic network, which must be broken for the mucus to act as a liquid, reforms quickly. Additional evidence of this process is provided by two further tests.

1 After a sample had been deformed a number of times at a given shear rate, the sample was allowed to rest for 10-12 seconds while the controls were set for another set of deformations at a new shear rate. It was noted that the initial deformation in this new series showed a considerably higher yield stress than subsequent deformations. The values of yield stress shown in Figure 4.16 are for deformations subsequent to the initial deformation as these

are more characteristic of the properties of mucus under a moving slug. Figure 4.18 shows values from a typical sample comparing initial breaking stress (σ_{yi}) and subsequent (σ_y) values for yield stress as a function of shear rate. It is apparent that the elastic network formed when the mucus rests for 10-12 seconds is stronger than that formed when rest is allowed for only one second.

The time course of the healing process was examined through another series of tests using the cone and plate apparatus as shown in Figure 4.19. In these tests one complete cycle of deformation was performed on the sample. The sample was then allowed to "heal" for varying lengths of time. After healing the sample was again stressed, but only to a sub-yield level. Rotation of the plate was then halted and a stress relaxation time (τ) measured. As a matter of convenience, τ was chosen as the time (in seconds) required for the stress to relax to 0.20 of the initial stress (σ_0 in Figure 4.1). The results of a typical test are presented in Figure 4.20. Relaxation times for any given heal time proved to be quite variable. However, the general trend of increasing τ with increasing heal time is apparent and is taken as evidence that the sample becomes more solid the longer it is allowed to heal. This property of the healed mucus can be compared to the mucus in its fluid form. When the rotation of the plate is stopped while the mucus is in its fluid form, the force decays quickly to zero. The relaxation time for this decay

FIGURE 4.18. The initial yield stress after a sample has been left unstressed for a period of time is greater than subsequent yield stresses after only short term "heal" periods.

Figure 4.18

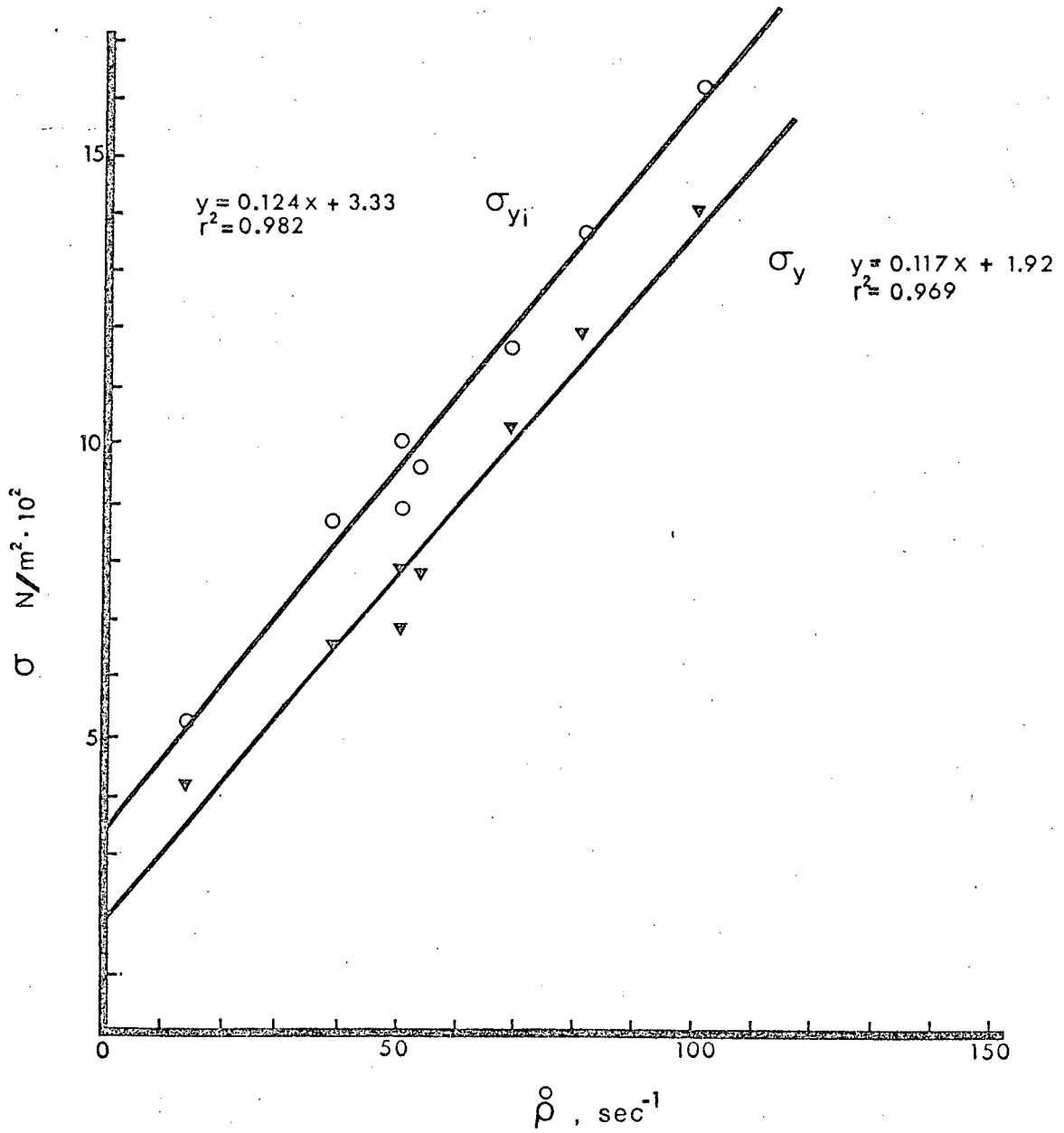


FIGURE 4.19. The testing procedure used to determine the effect of heal time on the recovery of solidity. Relaxation time, here defined as the time required to relax to 0.20 of the initial stress, is used as a measure of solidity.

Figure 4.19

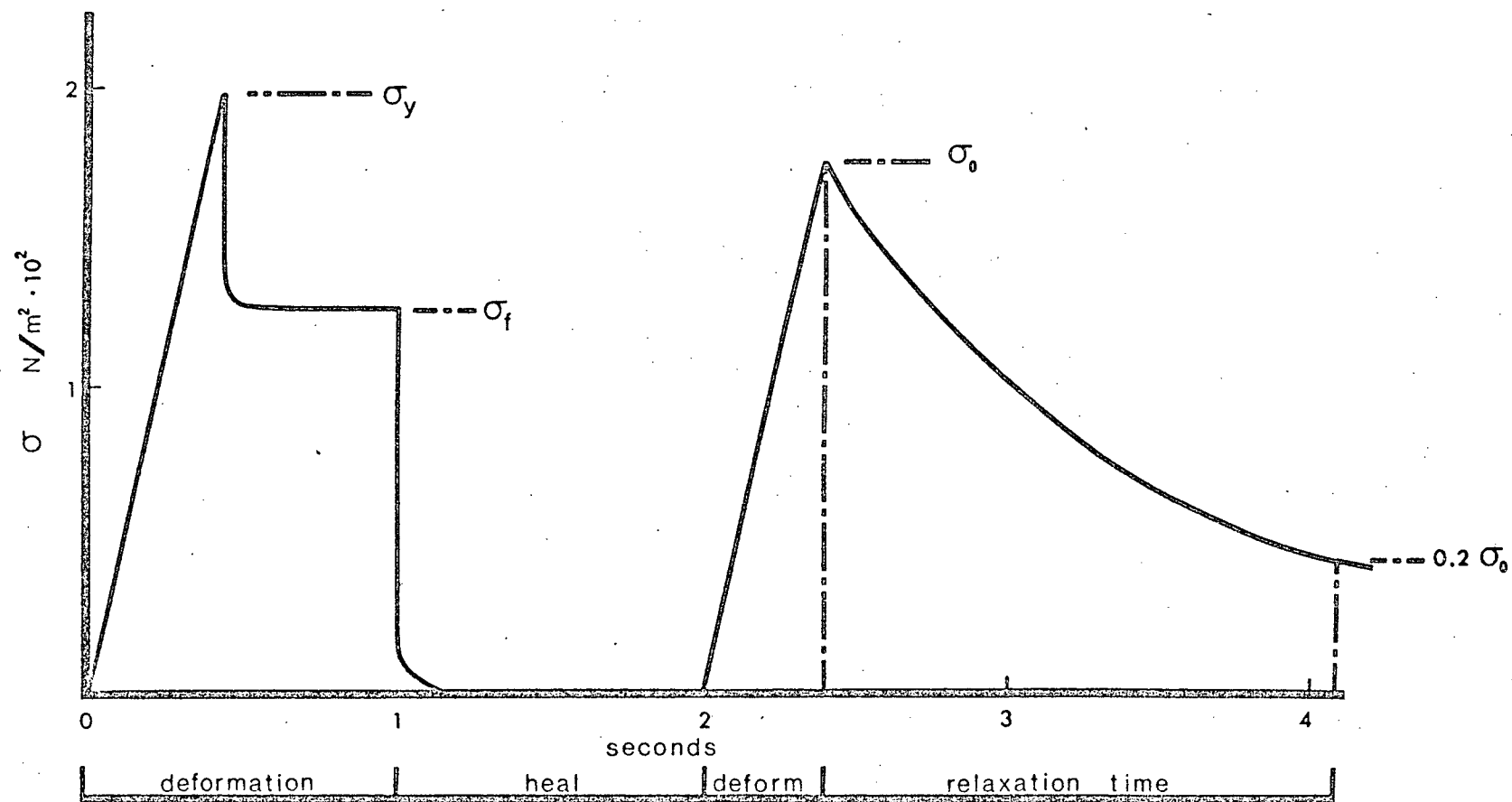
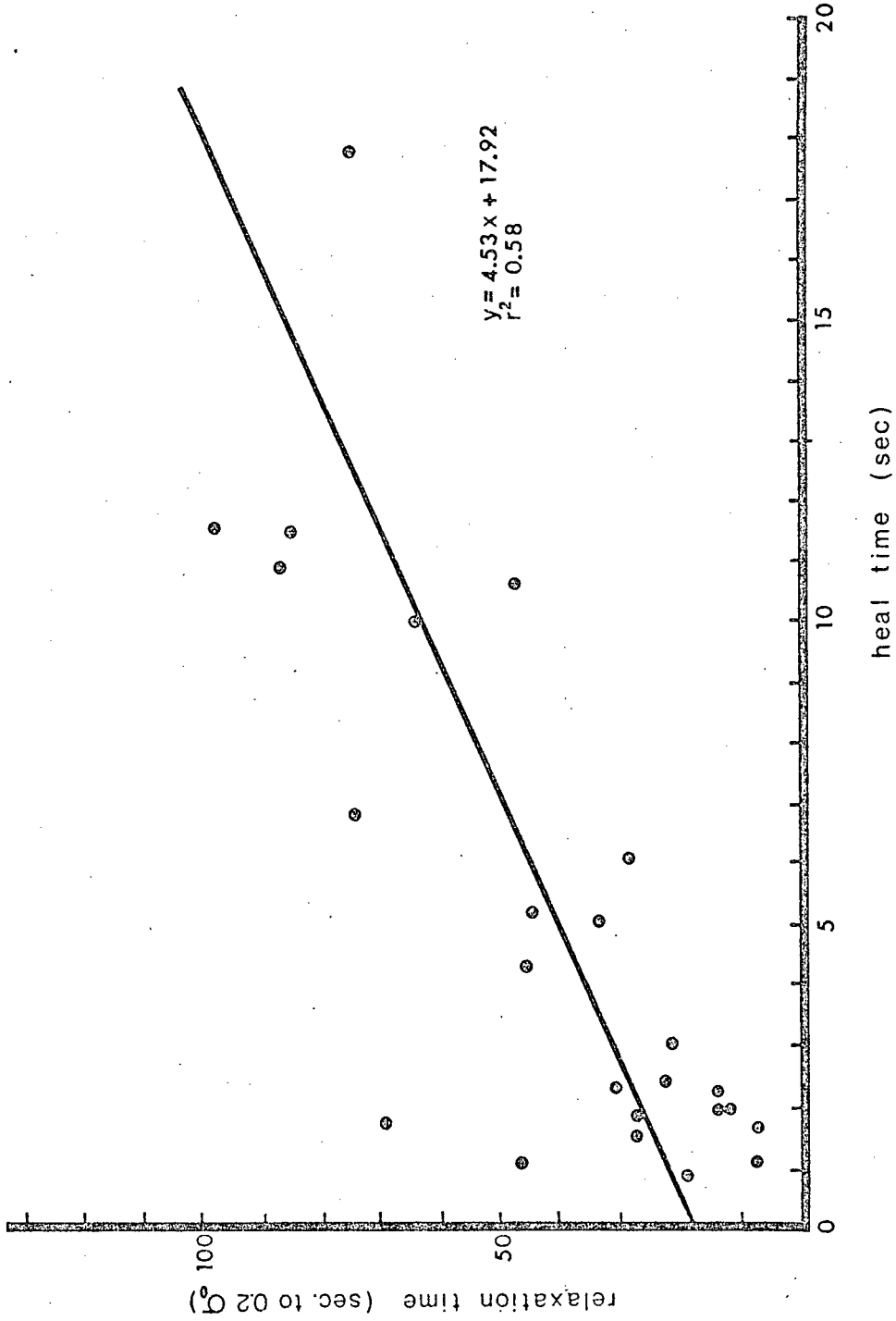


FIGURE 4.20. A representative plot of relaxation time versus heal time for Ariolimax columbianus pedal mucus. The range shown in these tests was:

$$\begin{array}{lll} \text{low} & y=0.11x + 0.41 & r^2=0.71 \\ \text{high} & y=5.75x + 27.59 & r^2=0.51 \end{array}$$

Figure 4.20



is too short to be accurately measured by this testing procedure but is certainly less than 0.1 seconds. Thus it can be seen that the mucus recovers considerable solidity in a period of less than a second.

The physical properties of A. columbianus pedal mucus can be summarized as follows:

1) At shear ratios less than 5-6 the mucus behaves as a viscoelastic solid. The shear modulus is on the order of 100 to 1000 N/m², increasing with increasing shear rate.

2) The mucus shows a sharp yield point at a shear ratio of 5-6. Yield stress increases with increasing shear rate.

3) At a shear ratio of greater than 6 the mucus behaves as a fluid with a viscosity of 30-50 poise.

4) The ratio of yield stress to shear rate for any one sample is about 2.0

5) The fluid mucus can recover its elasticity if allowed to heal for a period of time.

6) The amount of solidity recovered increases with increasing time.

Fiber Formation

The properties summarized above were measured under conditions designed to simulate those beneath a walking slug. However, being lethargic beasts, slugs spend large periods of time simply sitting in one spot. In the cages used to house the slugs for this study the preferred resting position for A. columbianus was halfway up the vertical glass walls. The slugs commonly spent periods of 12-24

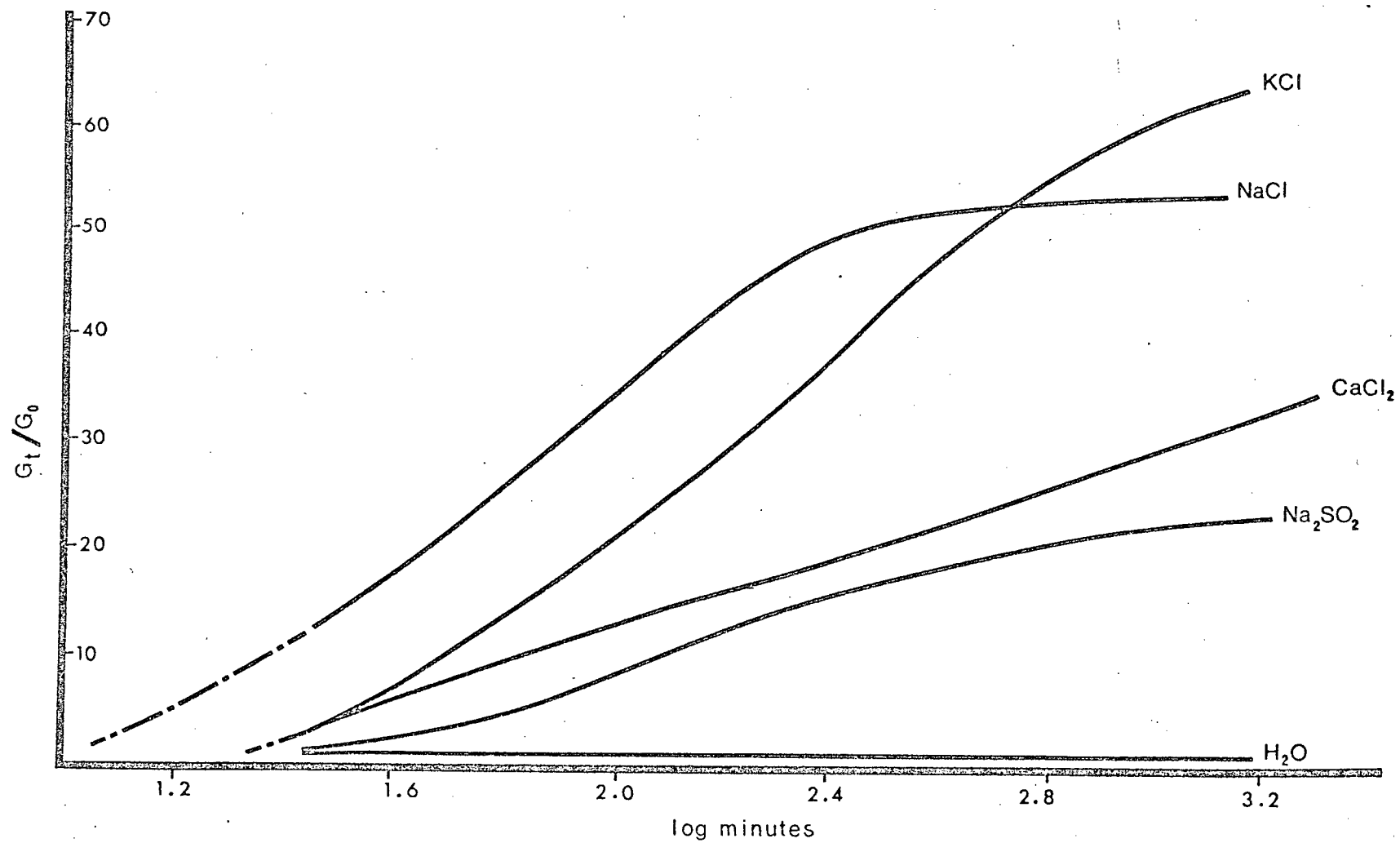
hours thus attached. Since it has been shown by stress relaxation tests that pedal mucus will flow over long periods of time, why do slugs attached to vertical walls not gradually slide down under the force of gravity? The answer to this question may lie with another property of pedal mucus, its ability to form fibers.

If a slug that has been attached to a vertical wall is gently pried off, a white layer of mucus will often remain behind. Upon examination under a polarizing microscope the mucus is found to contain, in addition to the usual debris, a dense feltwork of fibres. It is difficult to trace a single fiber from end to end but they appear to be quite long (up to about .5mm). Fibres are about 1.0 μm in diameter and are weakly birefringent. The fibres do not dissolve if the sample is placed in distilled water. The time course of fiber formation beneath a resting slug has not been studied.

It was found possible to induce fibre formation in pedal mucus in the dynamic testing apparatus. Mucus alone (at 100% relative humidity) either does not form fibers or fibers are formed too slowly to be detected in these tests. If, however the sample is immersed in a salt solution, fibers rapidly form. The formation of fibers is accompanied by a dramatic increase in the shear modulus of the sample. This modulus was measured by stress-shear ratio tests at shear ratios less than 0.10 and shear rates less than 0.10/sec. Figure 4.21 shows the results of one series of tests; following the time course of this increase in modulus

FIGURE 4.21. The effects of various salts on the relative increase (G_t/G_o) in shear modulus due to fiber formation in Ariolimax columbianus pedal mucus. All salts were present as 0.05 M aqueous solutions.

Figure 4.21



for various solutions. An insufficient number of tests were performed to be able to attribute the different time courses found as being a result of the different salts applied. It can be concluded, however, that while fiber formation is dependent on the presence of salt (no fibers being formed in distilled water) the process is not dependent on either a specific cation or anion, or the valence of either the cation or anion. Again, once fibers were formed they would not dissolve if the sample was exhaustively dialyzed against distilled water and the salts thereby removed.

In order to predict the behavior of fibrous mucus under a resting slug it would be necessary to perform "creep" tests where the sample is subjected to a constant stress and the deformation measured as a function of time. Unfortunately machines that allow for creep tests to be performed in shear (as would be necessary for mucus) are difficult to design and construct. Consequently no attempt has been made in this study to test the creep characteristics of fibrous and non-fibrous pedal mucus. It is possible however to make an educated guess as to what these properties might be.

It may reasonably be assumed that when fibers form in the pedal mucus under a slug some, but not all, of the glycoprotein chains are bound into fibers. The birefringence of the fibers indicates that their molecular structure is ordered. Without exception all other biological birefringent fibers have a modulus considerably higher than that of the randomly arranged mucus network.

No evidence can be seen under the microscope that the fibers are connected. Thus it seems reasonable that this fibrous mucus consists of a low modulus matrix (the mucus elastic network) through which run discontinuous higher modulus fibers. Another class of biological materials with a very similar structure - sea anemone mesoglea - has been studied by several authors, notably Gosline (1971a and b) and Koehl (1977a and b). The relevant findings of these authors are as follows: 1. The modulus of a discontinuous fiber reinforced composite such as mesoglea increases as the proportion of fibers to matrix increases. 2. For a given rate of deformation of the materials as a whole the presence of fibers serves to increase the shear rate acting on the viscous component. As a consequence a fiber reinforced material creeps more slowly than one that is not reinforced.

It has been shown that the modulus of fibrous mucus increases as one would expect if the material were to behave analogously to mesoglea. It can thus be guessed that the fibrous mucus will creep more slowly than the nonfibrous slime. If this is indeed so, it could explain the ability of slugs to remain attached to vertical walls without slipping.

CHAPTER FIVE

Chemical Composition

As shown in the preceding chapter A. columbianus pedal mucus is a viscoelastic material with some unusual properties. On the basis of these properties the existence in the mucus of a network of macromolecules has been hypothesized. Before it is possible to more closely examine the nature of this network it is necessary to identify the chemical composition of the building blocks from which this network is constructed. Just as the size, shape, and strength of bricks determines the possibilities for the overall form of a building, the chemical properties of individual monomers set the limits for the form and strength of polymer networks. Consequently the chemical analysis of pedal mucus forms the subject of this chapter.

What Is Mucus?

The term "mucus" has never been precisely defined. In general, any extracellular, viscoelastic animal secretion formed primarily of water is liable to be labelled "mucus". As such, the term is applied to a large variety of secretions, the functions of which range from locomotion, to feeding, to protection and reproduction (Hunt, 1970; Gottschalk, 1972). Given this broad functional diversity it is somewhat surprising to find that most, if not all, mucins have a basically similar chemical composition. While there are many variations on the theme, all mucins studied

to date consist of some sort of complex between a polysaccharide and a protein (Hunt, 1970); this being dissolved in water. These polysaccharide-protein complexes fall into two general categories. If the composition of the complex is dominated by the polysaccharide, the complex is termed a mucopolysaccharide. If, on the other hand, the protein dominates, the complex is termed a glycoprotein. In general, in a mucopolysaccharide the polysaccharide and protein are not covalently bound while in a glycoprotein they usually are (Hunt, 1970).

Mucus Collection

Mucus was collected from healthy A. columbianus as described in Chapter 3. For chemical analysis the mucus from approximately twenty slugs (about 5 ml) was pooled and divided into two aliquots. The first of these was immediately frozen at -80 °C and lyophilized to provide a sample of the whole mucus as it appears on the foot of the slugs. The second aliquot was dialyzed against six one liter changes of distilled water over a period of three days to remove any unbound small molecular weight molecules. After dialysis this aliquot was frozen at -80 °C and lyophilized. Both lyophilized samples were stored dessicated at room temperatures.

Analysis

Water Content

Mucus was collected from eighteen slugs and immediately weighed. The samples were then dried at 105 °C for twenty-four hours and reweighed. The dry weight (expressed as a percent of the wet weight) averaged 3.44% and ranged from 2.85 to 4.46%. Thus pedal mucus is about 95 to 97% water.

Protein

The protein content of pedal mucus was measured by the heated biuret-folin assay of Dorsey, McDonald, and Roels (1977) using bovine serum albumin (Sigma) as a standard. This assay is preferred over that of Lowry *et al.* (1951) in that it provides an estimate of protein content that is not biased by the amino acid composition of the protein being tested. Assays were run in triplicate and the values presented are the means.

Whole pedal mucus was found to contain 33.6% protein (weight of protein/dry weight of mucus) while the dialysed sample contained 45.6% protein. The increased proportion of protein in the dialyzed sample is presumably due to the loss of small, nonprotein molecules (primarily salts) during dialysis.

The amino acid composition of the pedal mucus protein was determined using a Beckman 119C amino acid analyzer. Samples were hydrolyzed with 6 N HCl *in vacuo* for 24 hours at 100 °C. Standards and samples were chromatographed using a three hour sodium citrate buffer cycle described in Beckman Operating Notes (119C AN001, 1975). The chromatograms were tabulated by standard procedures. Three

samples of each aliquot were analyzed and the averaged results are presented in Table 5.2.

The amino acid composition is noteworthy in three respects.

1. If it is assumed that the glutamic and aspartic acid residues measured represent glutamic and aspartic acids present in the protein (rather than asparagine and glutamine) , the acidic amino acids form a large proportion of the total. Where the basic amino acids (lysine and arginine) comprise only about 4% of the total number of amino acids the acidics comprise 17 to 20% . If this assumption is valid, at physiological pHs the protein will have a net negative charge due to the dissociated carboxyl group of the aspartic and glutamic acids and will behave as a polyanion. The polyelectrolytic behavior of the mucus as a whole is confirmed in the next chapter.

2. Cysteine is present in the mucus protein. The sulfhydryl group of one cysteine side chain can be oxidized by the sulfhydryl of another cysteine to form a disulfide bond between the two yielding a cysteine molecule. Such disulfide bonds are a common method for crosslinking protein chains and thus are a likely candidate for forming the crosslinks of a polymer network. It will be shown in the next chapter that disulfide bridges, presumably formed by cysteine, do indeed crosslink the network of A. columbianus pedal mucus.

3. Serine and threonine are present in large amounts (23-24%). These two amino acids are likely sites for the

Table 5.1: Amino Acid Composition (residues/100 residues)

	whole	dialyzed
Aspartic acid	9.8	9.0
Threonine	11.7	11.1
Serine	12.2	12.6
Glutamic acid	10.9	8.4
Proline	8.0	8.6
Glycine	9.3	8.6
Alanine	7.4	7.4
Half Cystine	0.6	3.0
Valine	5.2	5.2
Methionine	trace	0.2
Isoleucine	4.5	4.5
Tyrosine	1.7	1.8
Phenylalanine	2.3	2.3
Lysine	1.1	0.9
Histidine	3.7	3.4
Arginine	3.0	3.9
Leucine	5.5	5.5
<u>Acidics</u>	20.7	17.4
<u>Basics</u>	4.1	4.8

covalent bonding of polysaccharide to the protein chain (Gottschalk, 1972). The existence of these bonds will be demonstrated in the next chapter.

Polysaccharides

No simple assay exists that unambiguously measures the total sugar content of a polysaccharide. Consequently the pedal mucus was assayed separately for the various sugars and sugar derivatives that were likely to be present.

Uronic Acids

The total content of uronic acids was measured using the assay of Blumenkrantz and Osboe-Hansen (1973) using glucuronic acid (Sigma) as a standard. Assays were performed in triplicate and values averaged. The results show that whole pedal mucus contains 6.8% (wt/wt) uronic acid and dialyzed mucus, 7.7% (wt/wt). No attempt was made to further define the identity of the uronic acids. As with the glutamic and aspartic acids of the protein, the dissociated carboxyl groups of the uronic acids will cause the mucus to act as a polyanion. The mucus does indeed show the characteristics of a polyanion as will be shown in the next chapter.

Amino Sugars

The total content of amino sugars was measured by the assay of Boas (1953) using galactosamine (BDH) as a standard. Samples were hydrolyzed in 2N HCl in vacuo for 20

hours at 100 °C. Tests were performed in triplicate and values averaged. The amino sugar content of the whole mucus was 6.9% (wt/wt) while that of dialyzed samples was 7.8%.

Aliquots of the eluate from the Dowex 50 column of the assay were chromatographed on a Beckman 119C amino acid analyzer using a 450 minute lithium citrate cycle described in Beckman Operating Notes (119C AN004, 1975). Glucosamine and galactosamine (BDH) were used as standards. The results show that the amino sugars in pedal mucus are primarily glucosamine (6.1% whole mucus, 6.9% dialyzed mucus wt/wt) accompanied by a small amount of galactosamine (0.8% whole mucus, 0.9% dialysed mucus wt/wt) . There is a strong possibility that these amino sugars are present in the mucus as N-acetyl amino sugars the acetyl groups being removed during hydrolysis. The fact that the acetamido bond is as susceptible to acid hydrolysis as the glycosidic bonds (which must be broken to release the monosaccharide for assaying) makes it extremely difficult to assay for the presence of N-acetyl hexoses (Marshall and Newberger, 1972). However, in virtually all cases in which the presence of N-acetyl groups has been tested they have been found (see Table 5.3). One further fact lends support to the suggestion that the amino sugars are acetylated : if the amino groups of the amino sugars are not acetylated (or otherwise bound) they will be charged at physiological pH's. These positive charges will tend to offset the negative charges of the uronic acids. As a consequence the polyanionic character of the mucus should be decreased.

Since, as will be shown in the next chapter, the mucus acts as a strong polyanion, it is likely that the amino groups of the amino sugars are acetylated, and therefore probably not able to ionize.

Sialic Acid

The total sialic acid content was measured using the H₂SO₄ hydrolysis and the thiobarbituric acid assay of Warren (1959). N-acetylneuraminic acid (Sigma) was used as a standard. Assays were performed in triplicate. There is no detectable sialic acid present in pedal mucus.

Neutral Sugars

The total content of neutral sugars was measured by the phenol-sulfuric acid assay of Lo, Russel, and Taylor (1970) using glucose (Sigma) as a standard. While this assay preferentially measures neutral sugars, it also measures uronic acids (but not amino sugars). Consequently, the results from this assay were corrected using the results from the uronic acid assays. Tests were performed in triplicate and averaged. The results show that whole mucus is 7.2% (wt/wt) neutral sugars, and dialyzed mucus, 8.5% wt/wt.

The composition of the neutral sugars was further examined using a gas/liquid chromatograph. Samples of whole and dialyzed mucus were hydrolyzed, acetylated, and chromatographed using the method of Court (1978). The results show that the neutral sugars of pedal mucus consist

primarily of fucose (3.4 to 5.6%) and galactose (2.3 to 3.2%) accompanied by small amounts of glucose and mannose (see Table 5.2).

Sulphated Sugars

Many invertebrate mucins contain sulphated polysaccharides (Hunt, 1970) . The possible presence of sulphated sugars in pedal mucus was examined by measuring the total sulphate content, using both methods of Dodgson and Price (1962) and Nader and Dietrich (1977). Both these assays measure only free sulphate. To liberate any sulphate bound to sugars, the mucus samples were hydrolyzed using either 8 M HCl (6 hours, 100 C) (Nader and Dietrich, 1977) or 25% formic acid (24 hours, 100 C) (Antonopoulos, 1962). All assays were performed in triplicate using potassium sulphate as a standard. The results of both assays show that there is no detectable sulphate present in

A. columbianus pedal mucus.

In addition to sulphate the method of Nader and Deitrich (1977) measures any phosphate present. However, no phosphate was detected.

Salts

The composition of the cations present in pedal mucus was analyzed using a Techtron AA20 atomic absorption flame spectrophotometer. All preparations for the assays were performed using polypropylene test tubes and pipettes. All assays were carried out in quintriplicate and the results

Table 5.2: Chemical Composition

	<u>whole</u>	<u>% weight</u>	<u>dialyzed</u>
Glucosamine	6.1		6.9
Galactosamine	0.8		0.9
Neutral Sugars	7.2		8.5
fucose	3.4		5.6
galactose	3.2		2.3
mannose	0.4		0.4
glucose	0.2		0.3
Uronic Acid	6.8		7.7
Sialic Acid	0.0		0.0
<u>Total Carbohydrate</u>	20.9		24.0
<u>Protein</u>	33.6		45.6
<u>Salts</u>			
$\text{SO}_4^{=}$	0.0		0.0
Na^+ (as the chloride)	2.5		1.3
K^+ (as the chloride)	9.2		1.9
Mg^{++} (as the chloride)	3.6		2.6
Ca^{++} (as the chloride)	0.6		0.6
<u>Total</u>	15.9		6.4
<u>Total</u>	70.4		76.0

averaged. Results were calculated using standard curves generated at the same time as samples were assayed. The results are presented as the weight of the chloride salt of the cation, expressed as a percent of the total weight of the mucus. The specifics for the assay of each cation are as follows:

Sodium: Weighed samples of lyophilized mucus and standards were dissolved in deionized, glass distilled water. Sodium chloride was used as a standard.

Potassium: Weighed samples of lyophilized mucus and standards were dissolved in deionized distilled water containing a swamp of 500 parts per million sodium. Potassium chloride was used as a standard.

Magnesium: Weighed samples of lyophilized mucus and standards were dissolved in deionized distilled water containing 1.5% EDTA. Magnesium chloride was used as a standard.

Calcium: Weighed samples of lyophilized mucus and standards were dissolved in 0.1 M HCl containing 0.5% LaCl₃. Calcium chloride was used as a standard.

All assays were carried out using the wave lengths and slit widths specified in the AA20 operating manual.

The results are presented in Table 5.2. These results are noteworthy in two respects:

1. While some of the ions are lost during dialysis, nearly half remains bound to either the protein or carbohydrate of the mucus. Presumably these ions are electrostatically bound to the dissociated carboxyl groups

of the glutamic, aspartic and uronic acids.

2. Potassium ions are lost preferentially to sodium ions, and magnesium to calcium. Presumably this is due to sodium and calcium being preferentially bound. The basis for this effect is not known.

The anionic composition of pedal mucus was not analyzed.

The chemical analysis presented here is far from exhaustive and accounts for only 76% of the weight of the glycoprotein. The reason for this incomplete recovery is not precisely known. Some of the remainder may be lipids, for which this study has not tested. Unexpected losses during various hydrolyses may also account for some of the unrecovered weight. Much more work will be needed to define the precise chemical composition of this mucin. However, for the purposes of the present study, this preliminary examination is sufficient. In summary, the significant aspects of the chemical composition are as follows:

1. The mucus is 95 to 97% water the remainder being salts and a protein-polysaccharide complex.

2. Protein contributes the larger portion of the protein-polysaccharide complex; as such the mucin is classed as a glycoprotein.

3. Amino acid analysis reveals that the protein is likely to be a polyanion.

4. The carbohydrate portion of the protein polysaccharide complex is evenly divided among neutral sugars, amino sugars, and uronic acids.

5. No sialic acid was detected.

6. The uronic acids contribute to the polyelectrolytic nature of the mucus.

7. Neither sulphate nor phosphate was detected in the mucin.

Comparison To Other Mucins

On the basis of these findings, the chemical composition of A. columbianus pedal mucin can be compared to and contrasted with other mucins.

Vertebrate Mucins

Ariolimax columbianus differs from most vertebrate mucins (such as tracheal and cervical mucins) in that it does not contain sialic acid. The absence of sialic acid in invertebrates is virtually catholic as noted by Warren (1963). In this respect the polysaccharide of pedal mucus bears similarity to a polysaccharide found in both vertebrates and invertebrates - hyaluronic acid. The basic unit of hyaluronic acid is a disaccharide consisting of a glucuronic acid coupled (through a B glycosidic linkage) to an N-acetyl glucosamine. Pedal mucus differs from this by also containing neutral sugars. However, since the manner in which the monosaccharides of pedal mucus are linked is unknown the precise differences between it and hyaluronic acid are unknown.

Invertebrates

Ariolimax columbianus pedal mucus differs from invertebrate mucins in general in that it does not contain sulphated sugars. In this respect it specifically differs from most gastropod mucins which have been studied. The most detailed analysis to date of gastropod pedal mucus is that of the North African land snail, Otella lactea (Pancake and Karnovsky, 1971). They found that this mucus contained glucosamine (probably acetylated and sulphated) and iduronic acid. The ratio of uronic acid to hexosamine was 1.14 to 1. No neutral sugars were detected. This is in contrast with the study of Suzuki (1941) who found that the pedal mucus of Helix laeda contained galactosamine (probably acetylated) and galactose, but neither sulphate nor uronic acids. Neither Pancake and Karnovsky nor Suzuki examined the amino acid content of the mucus secretions. The chemical composition of various mucins are compared to A. columbianus pedal mucus in Table 5.3.

Other studies of gastropod mucus secretions have been generally confined to the hypobranchial mucins of whelks (as reviewed by Hunt, 1970). These secretions all contain sulphated polysaccharides. The protein component of at least one of these secretions, that of Buccinum undulatum (Hunt and Jevons, 1965) bears a striking similarity to the protein of A. columbianus pedal mucus (see Table 5.3).

This comparison of pedal mucus to other mucins is of limited use for a number of reasons. First, so little research has been carried out concerning the distribution of the various chemical components of mucus that it is

Table 5.3: The Carbohydrate Composition of Various Proteoglycans and Glycoproteins

Polysaccharide	Occurrence (vertebrate or invertebrate)	Components		N-acetyl	Sulphate
		Repeating	Others		
hyaluronic acid	both	D-glucosamine D-glucuronic acid	L-arabinose (?) D-galactose (?) D-glucose (?)	+	-
chondroitin	both	D-galactosamine D-glucuronic acid	D-xylose D-galactose	+	-
chondroitin 4 or 6 sulphate	both	D-galactosamine D-glucuronic acid	D-xylose D-galactose	+	+
dermatin sulphate	both	D-galactosamine L-iduronic or D-glucuronic acid	D-xylose D-galactose	+	+
heparin	both	D-glucosamine D-glucuronic or L-iduronic acid	D-xylose D-galactose	-(+)	+
skeletal keratin sulphate	both	D-glucosamine D-galactose	D-galactosamine L-fucose Sialic acid D-mannose	+	+
glycan sulphate	both	glucose or fucose or galactose			
ovine or bovine submaxillary mucin glycoprotein	vertebrate		galactosamine sialic acid D-galactose L-fucose	+	-
pig or human gastric mucin glycoprotein	vertebrate		glucosamine galactosamine galactose fucose sialic acid	+	-

Table 5.3: (continued)

Polysaccharide	Occurrence (vertebrate or invertebrate)	Components		N-acetyl	Sulphate
		Repeating	Others		
<u>Otella lactea</u> pedal mucus (a)	invertebrate		glucosamine hexuronic acid(s)	+	+
<u>Helix laeda</u> pedal mucus (b)	invertebrate		glucosamine galactose	+	-
<u>Buccinum undatum</u> hypobranchial mucus(c)	invertebrate		glucosamine galactosamine galactose mannose fucose glucose	+	-
<u>Ariolimax columbianus</u>	invertebrate		glucosamine galactosamine fucose galactose mannose glucose uronic acid(s)	+(?)	-

(a) Pancake and Karnovsky (1971)

(b) Suzuki (1941)

(c) Hunt and Jevons (1965)

Table 5.3: (continued) A comparison of the amino acid composition of slug pedal mucus and whelk hypobranchial mucus.

Amino acid	<u>Ariolimax columbianus</u>	<u>Buccinum undatum</u> (c)
aspartic acid	9.0	11.10
threonine	11.1	6.45
serine	12.6	6.27
glutamic acid	8.4	11.34
proline	8.6	5.19
glycine	8.6	5.19
alanine	7.4	7.67
cystine	3.0	1.73
valine	5.2	6.91
methionine	0.2	0.31
isoleucine	4.5	4.20
leucine	5.5	8.16
tyrosine	1.8	2.85
phenylalanine	2.3	3.66
lysine	0.9	7.29
histidine	3.4	2.45
arginine	3.9	5.54

impossible to say with any certainty what is and is not "typical". While A. columbianus pedal mucus appears to differ in significant aspects from other mucins, it seems likely that this reflects a bias in the historical choice of the secretions studied, rather than some unique quirk of slug biochemistry.

Second, while mucous secretions as a whole have received scant attention, invertebrate epithelial mucins have received even less attention. The vast preponderance of the present knowledge of these mucins is based solely on histochemical studies. Hopefully in the future the broad distribution and importance of invertebrate mucins will be recognized and this situation rectified.

CHAPTER SIX

Physical Chemistry

Armed with the knowledge of the chemical composition of pedal mucus, it is now possible to examine the elastic network of A. columbianus mucus in more detail. Specifically it will be useful to examine the following questions concerning the network and the molecules from which it is constructed.

1. How large are the molecules that make up the network?
2. Is the network constructed from one type of molecule, or are different types joined together?
3. How does the glycoprotein, which forms only 3 to 4% of the hydrated mucus, influence the water around it?
4. How are the individual molecules crosslinked to form a network?
5. And finally, once this network is broken apart during locomotion, how does it manage to reconstruct itself?

With these questions in mind, a number of tests were performed. Each test provides information concerning several of these questions. Taken together, these tests provide enough information to answer some of these questions and thereby construct a reasonable picture of the macromolecular construction of A. columbianus pedal mucus. A complete answer to these questions must, however, wait until further tests are carried out.

Before describing the testing procedures of this study it will be useful to explain the concepts and terms used when dealing with the physical chemistry of polyelectrolytes.

Polyelectrolytes

As explained in Chapter 4, a flexible polymer chain in solution will assume a random configuration. A number of mechanisms may be used to alter this configuration. All of these mechanisms involve the action of a force on the chains; this force supplying the energy to impose order on the randomly configured chains. One such mechanism, the crosslinking of the chain to other chains, and the subsequent application of an external force, has already been described. Another mechanism which may affect the shape of a polymer chain is the interaction of charges present on the chain.

Imagine a polymer chain such as that depicted in Figure 6.1a. Every third link of this chain contains a dissociated carboxyl group and therefore a negative charge. Any such polymeric macromolecule containing many charged groups is a polyelectrolyte. In this case it is a polyanion. Negative charges repel each other, the force of repulsion decreasing with distance. As a consequence the negative charges on the chain links are forced apart. Each charge moves away from its neighbors, thereby maintaining a minimum energy configuration. If this were the only factor operating, this charge repulsion would cause the polymer chain to extend

- FIGURE 6.1. The characteristics of polyelectrolytes.
- A) If the charged chain were randomly configured (high entropy) many of the charges would be close together.
 - B) The charges are a maximum distance apart when the chain is fully extended. However, the entropy is low.
 - C) A real polyelectrolyte chain represents a compromise between A. And B.
 - D) The presence of counterions can mask charges from each other, allowing the chain to assume a more compact and random configuration.

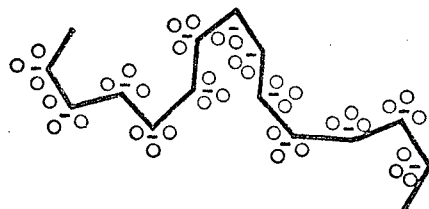
FIGURE 6.1

PolyelectrolytesA Random Coil

high entropy
high charge density

B Rod

low entropy
low charge density

C CompromiseD Charge Masking

o - positive
charge

into a long rod, a very ordered configuration (see Figure 6.1b). As with a neutral polymer, however, thermal agitation will cause the polymer chain to contort. The final shape of the chain will be a compromise between the extended rod expected for minimum electrostatic energy and the random coil favored by thermal agitation (see Figure 6.1c).

If this polymer chain, rather than existing on its own, is crosslinked to similar chains to form a network, the electrostatic interactions described above will cause the network to repel itself and expand to a larger volume than if the charges were not present. This is the situation that should occur if a crosslinked polyanion is dissolved in distilled water. This situation will be changed, however, if a salt (eg. NaCl) is added to the water, the dissolved salt will be electrostatically attracted to the negative charges of the polyanion, and a cluster of cations will form around each negative charge, as shown in Figure 6.1d. These counterions will serve to mask the negative charges from each other, and the force of repulsion between neighboring links will decrease. Less energy is then available to impose order on the chain. In response the individual molecules will assume a more kinked configuration and the network will shrink somewhat. While the presence of counterions causes the force of repulsion to decrease it does not abolish the repulsion altogether. Consequently the network, in the presence of counterions, is still expanded above its uncharged level.

Precise explanations for the effects of charge density

and counterion density on the shapes of macromolecules may be found in the reviews by Katchalsky (1964), Veis (1970), or Tanford (1961).

Tests

Network Swelling

As shown in the preceding chapter, several of the chemical components of A. columbianus pedal mucus should be negatively charged at physiological pH's. If these components are linked together into polymer chains, they should show the characteristics of a polyanion as described above. This can be detected quite simply. If a sample of mucus is collected as described in Chapter 4 it forms a compact blob. If this blob is placed in a 0.1M NaCl solution (roughly equivalent to the salt concentration of natural mucus) it will remain roughly the same size and shape for several days. If however a sample (0.1 to 0.3 ml) of mucus is placed in 10 ml of distilled water the counterions diffuse out of the mucus and the blob will slowly expand to several times its initial size. Alternatively, the vast majority of the charges can be removed altogether by lowering the pH of the mucus blob below the level where the carboxyl groups of the glycoprotein are dissociated. The pH at which the carboxyl groups are half dissociated (the pK) is about 4. Thus when the pH is lowered below 4 most of the carboxyl groups will be undissociated and the mucus network, no longer held

expanded by the negative charges, should contract. This is indeed the case. A blob of mucus shrinks when placed in a solution of water the pH of which has been adjusted to 2.1 by the addition of HCl.

These swelling and shrinking effects can be easily quantified. As the network expands water is drawn into the interstices between the chains much as a sponge soaks up water. Conversely, the contraction of the network forces water out of the interstices. Consequently the amount of water contained in the network (expressed as hydration, milliliters of H₂O/gram of glycoprotein) is a measure of network expansion.

Hydration values were measured for A. columbianus pedal mucus in four solvents: distilled water, 0.1 M NaCl, 1.0 M NaCl, and water adjusted to pH 2.1 with HCl. The procedure was as follows: A small blob of mucus (0.05-0.15 ml) was placed in 25 ml of the test solution and allowed to expand or contract. All tests were conducted at room temperature (21-23 °C). Change in volume of the blob was visually complete within two hours, however the network was left in the solvent for 6-7 hours to ensure that equilibrium was reached. After equilibration the mucous blob was grasped with a forceps, removed from the solvent, and immediately weighed. Samples equilibrated with NaCl were then dialyzed against repeated changes of distilled water (24 hr., 22 °C) to remove the salt. All samples were then dried at 105 °C for 18 hours and reweighed. The value (wet weight-dry weight)/dry weight is the hydration expressed as grams of

water per gram dry weight. All tests were performed in quintuplicate. The results from these tests are presented in Table 6.1. These tests confirm the predictions made above. The mucus is highly sensitive to the composition of the solvent, being nearly 20 times as expanded in distilled water as compared to pH 2.1. In this fully expanded configuration nearly a liter of water is contained in the interstices of one gram of mucus network. Under conditions approximating those in natural mucus (0.1 M NaCl) the network is contracted to one fifth of its fully expanded size. If the equilibrium hydration value measured here for mucus in 0.1 M NaCl (206 g/g) is compared to that of freshly collected mucus (21-34 g/g) it is evident that mucus as it appears on the foot of the slug is not at equilibrium with its solvent. Presumably the mucus is secreted into the lumen of the suprapedal gland in a dehydrated state and the time spent on the foot is insufficient to allow equilibrium to be reached.

Solubility Tests

Anyone who handles a slug soon discovers that pedal mucus is very difficult to dissolve. Once a bit of slime attaches itself to your fingers, it seems that no amount of washing and scrubbing will make it go away. This simple fact is again tied to the presence in the mucus of a crosslinked network.

As explained in Chapter 4, an elastic network is elastic as a result of its being crosslinked. Further, a

Table 6.1: Hydration of Pedal Mucus

Solvent	Equilibrium hydration (ml H ₂ O gm dry mucus)	Estimate hydrodynamic hydration (ml H ₂ O/gm)
distilled water (pH=6.0)	954.7 ± 432.3	2430
0.1 M NaCl	206.1 ± 38.7	276
1.0 M NaCl	138.8 ± 24.4	197
pH 2.1	59.7 ± 12.6	28
Mucus as collected	21.4 34.1	

considerable deformation and stress must be imposed on the network before the crosslinks break and the molecules of the network can be pulled away from each other. A similar situation arises when the mucus network is placed in a large volume of fluid. The process of diffusion is such that the molecules of the network are energetically compelled to arrange themselves randomly throughout the fluid volume. This process is resisted by the crosslinks of the network. These crosslinks are of three possible types:

1. Protein or polysaccharide chains can be covalently bound to each other. These bonds are quite stable, requiring high temperatures, large forces, or chemical action to be broken.

2. Protein or polysaccharide chains can be bound to each other by "weak" bonds such as hydrogen bonds or hydrophobic interactions. These bonds are labile under conditions mild in comparison with those required to break covalent bonds. Further, at physiological temperatures the half life of an isolated weak bond may be quite short. As a result these bonds will be constantly rearranging. As a consequence it is possible that chains crosslinked by weak bonds can gradually be pulled apart under the influence of a small force. The short half life of a weak bond also ensures that if a bond is broken it can be reformed quickly.

3. Finally, protein or polysaccharide chains can be crosslinked by physical entanglements. Long polymer chains are likely to become entwined as they are jostled by thermal agitation. If two chains so linked are pulled upon it will

take some period of time for them to disentangle. During this period (while the chains are still entwined) the entanglements will act as crosslinks. Over longer periods, however, entanglements will become unraveled and thus cannot act as permanent crosslinks.

As long as any one of these forms of crosslink is present the mucous network will be maintained and the mucus will not dissolve. Only when the crosslinks are broken will the mucus be able to dissolve. Thus by examining the conditions under which the mucus dissolves, information may be gained concerning the type(s) of crosslink present in pedal mucus .

Before describing the experiments it is necessary to define the term "soluble". This is difficult to do because there exists a continuous spectrum of solubilities. At one end of the spectrum are small molecules which once in solution cannot be easily separated back out by "normal" means (filtration, centrifugation). At the other end of the spectrum are the macromolecules that are "colloidal" and can be separated by normal means. As with any continuous spectrum, solubility can be defined only by choosing a more or less arbitrary cut-off point. For the purposes of the present study such a point is chosen and a molecule is defined to be soluble in a liquid if:

1. It cannot be sedimented by low speed centrifugation (12,100 g for 30 minutes) and
2. It is not retained by a Nucleopore filter with a 1 μ m pore size.

Utilizing this operational definition, tests were conducted to determine under what conditions pedal mucus would dissolve. The test procedure was as follows:

Samples were collected as for the physical tests. A small volume of mucus (0.1 to 0.3 ml) was combined with ten ml of solvent in a Potter Elvehjem tissue grinder. The Teflon pestle of the grinder was then rotated at 1740 rpm and slowly inserted and withdrawn from the grinder tube. This stirring process was continued for five minutes and as a result the mucus was dispersed and the entire 10 ml sample became a transparent viscous liquid. After stirring samples were allowed to stand for 24 hours at either 25, 40, or 55 °C. The samples were then centrifuged at 12,100 g for 30 minutes. Formation of a gelatinous pellet was sufficient to show that the mucus had not dissolved. If a pellet did not form the sample was then filtered through a series of Nucleopore filters of descending pore size (5.0um, 1.0um, 0.8um, 0.6um, 0.4um, 0.2um, 0.1um). At some point in this series a pore size was reached at which the sample would completely occlude the pores and no more fluid could be forced through the filter. It was found that this cut-off point was usually quite distinct, a sample passing through all larger pore sizes with ease, before being completely retained by the final pore size. Some samples passed completely through a 0.1 um filter and this was noted.

A number of compounds known to be useful in dissolving macromolecules were tried and the results are shown in Table 6.2. The pore sizes shown are the smallest through which

Table 6.2: Solubility of A. columbianus pedal mucus

Solvent	25 C	40 C	55 C
distilled water	pellet	pellet	pellet
1% mercaptoethanol	pellet	0.1 μ m	0.1 μ m
8 M guanidine HCl	pellet	pellet	0.1 μ m
8 M urea	NF	5 μ m	0.1 μ m
formamide	5 μ m	5 μ m	pellet
2 M KCl	pellet	pellet	pellet
0.5% Triton X100	pellet	pellet	pellet
0.2% Tween 20	pellet	pellet	pellet

sizes noted are the smallest filter size the solution will go through.

NF=nonfilterable

the sample would pass.

It is apparent that at 25 °C none of the treatments solubilized the mucus in a period of 24 hours. However when the temperature is raised to 40 °C one compound effectively dissolved pedal mucus , 1% 2-mercaptoethanol. 2-mercaptoethanol contains a sulfhydryl group which reduces already existing disulfide bonds by forming a disulfide bond with the sulfhydryl of the cysteine molecules present in the mucus protein. Thus 2-mercaptoethanol acts by breaking protein-protein crosslinks. Subsequent tests showed that it was not necessary to disperse the mucus in order for 2-mercaptoethanol to show this solubilizing effect. A blob of pedal mucus placed in a 1% mercaptoethanol solution will, over the course of 24 hours at 40 °C, dissolve. The solubilizing effect of 2-mercaptoethanol and similar thiol reducing compounds (N-acetyl cystine, dithiothreitol) has been noted in studies dealing with several different types of mucin such as pig gastric mucin, and human cervical and bronchial mucins (see review by Creeth, 1978). When tests are carried out at 55 °C a number of compounds solubilize the mucus (see Table 6.2). All of these compounds are known to disrupt weak bonds such as hydrogen bonds or hydrophobic interactions. Thus, a second category of crosslink (in addition to S-S bonds) exists in the mucus. In this respect slug pedal mucus is again similar to such vertebrate mucins as pig gastric mucus and human cervical mucus. Unfortunately the precise nature of the weak bonds cannot be determined from these tests.

Intrinsic Viscosity Measurements

As explained in Chapter 4, viscosity is a measure of a fluid's resistance to the rate of deformation. Take for example the situation depicted in Figure 6.2a. A fluid is held between two plates. One plate is stationary and the second plate moves at a constant velocity. The fluid directly adjacent to each of the plates moves at essentially the same velocity as the plate itself (the "no slip condition"). As a consequence a velocity gradient is established in the fluid as shown in the figure. The fluid may be thought of as consisting of a stack of infinitesimally thick layers; each layer moving slightly faster than the next layer below it and slightly slower than the next layer above it. "Friction" between layers opposes the formation of this velocity gradient. The force needed to maintain this gradient is thus a measure of the internal friction (= viscosity, η) of the fluid. Now consider Figure 6.2b. A number of rigid particles have been added to the fluid. The plate moves at the same velocity as before hence the rate of deformation of the sample is the same. However, because a fraction of the volume contained between the plate is rigid (ie. non-deformable) the pattern of deformation within the fluid is changed. Each rigid particle will straddle several fluid layers. Since the fluid adjacent to each particle must move at the same speed as the particle the rigid particles will "tie" fluid layers together, disrupting orderly flow. This new pattern of flow requires the input of an additional force if it is to be maintained.

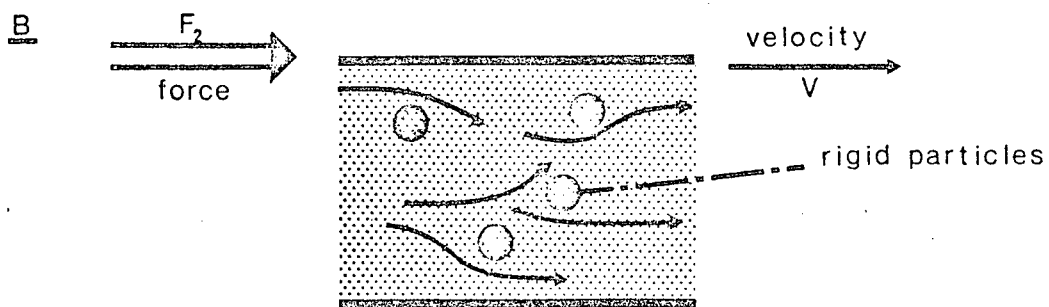
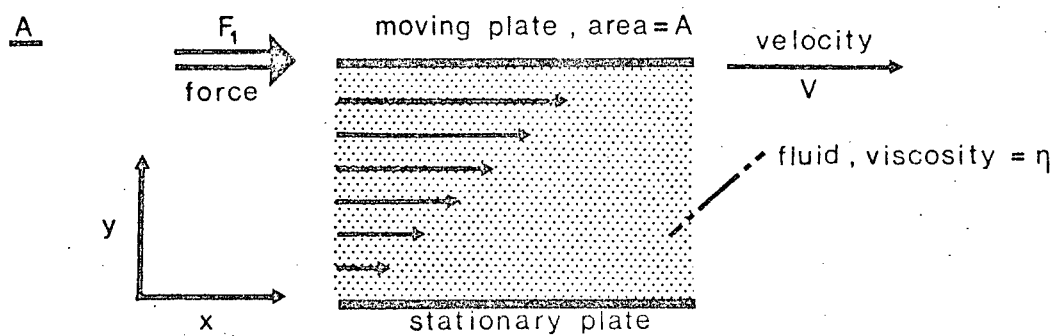
FIGURE 6.2. Viscosity

A) Fluid sandwiched between a moving plate and a stationary plate will establish an orderly velocity gradient. The force, F_1 , necessary to move the plate is

$$F = An(dx/dy)/dt$$

B) The presence of rigid particles in the fluid disrupts the orderly velocity gradient. As a consequence $F_2 > F_1$ even though the velocity of the plate and the viscosity of the fluid are not altered.

Figure 6.2



$$F_2 > F_1$$

Thus the internal friction of the fluid containing rigid particles is higher than that of the fluid itself and the apparent viscosity, n' , of the fluid containing rigid particles is higher than that of the fluid alone. The increase in apparent viscosity is a function of the concentration of particles, c (in grams/ml). The higher the concentration of particles the higher the apparent viscosity. It is possible to take this concentration dependence into account and arrive at a single number that describes the rigid particles' effect on the fluid. This is accomplished by measuring the apparent viscosity at a number of concentrations. Each apparent viscosity is then transformed to a specific viscosity, n_{sp} :

$$(n' - n) / n = n_{sp}.$$

A graph can then be drawn plotting the value n_{sp}/c (the reduced viscosity) against concentration. The extrapolation of these experimental points to zero concentration gives a certain value of reduced viscosity. This value is known as the intrinsic viscosity $[\eta]$, and has the units ml/g. Since it is measured at zero concentration the intrinsic viscosity can be thought of as a measure of the influence of a single particle on an infinite volume of fluid. It thus avoids any complications due to inter molecular interaction.

The intrinsic viscosity of a rigid particle is a function of both the shape of the particle and its effective hydrodynamic volume. The more a particle deviates from a

spherical shape, the greater its intrinsic viscosity will be. For rigid particles these two factors are related by the equation (Tanford, 1961):

$$\text{Equation 6.1} \dots\dots\dots [\eta] = s (v + h v_0)$$

where s is a measure of shape, v is the partial specific volume of the anhydrous particle (calculated to be 0.65 by Snary, Allen, and Pain (1970)), h is a measure of hydration (in ml H₂O/gm) and v_0 is the partial specific volume of the water which is carried with the particle as it moves (approximately 1.0). Note that this equation does not contain a term for the molecular weight. The independence of $[\eta]$ and molecular weight, M , holds only for rigid molecules. For a flexible molecule $[\eta]$ will vary with molecular weight such that

$$\text{Equation 6.2} \dots\dots\dots [\eta] = K M^{\alpha}$$

where K is an empirical constant and α is some value between 0.5 and 0.8. Alpha is usually determined empirically by procedures not applicable to mucus. As a starting point for further discussion it will be assumed here that the dispersed mucus is a solution of rigid hydrodynamic particles and that equation 6.1 is therefore applicable. It will be kept in mind however that a molecular weight dependence may cause these calculated values to deviate from reality. Equation 6.1 will be used

to provide a means by which the general shape and hydrodynamic volume of molecules may be examined. The equation is applied as follows: The value of s is at a minimum ($s=2.5$) when the molecule is a sphere. Thus

$$[\eta] = 2.5 (0.65 + h v_0) \text{ or}$$

$$h = 1.625 [\eta]/2.5$$

where v_0 is assumed to be equal to the density of bulk water ($=1.0$). This value for the hydration represents the maximum value for hydration. Conversely, it could be assumed that hydration is at a minimum ($h=0$) so that

$$[\eta] = 0.65 s \text{ or}$$

$$s = [\eta]/0.65$$

This will give a maximum value for the shape parameter. This parameter can be related to the relative dimensions of a spheroid (Simha, 1940). In reality the true values for h and s will lie somewhere between the maximum and minimum values calculated here.

The present study makes use of this theory by examining the intrinsic viscosity of particles formed from small fragments of the mucus network when mucus is dispersed by shearing in distilled water as described above. It seems reasonable to assume that particles of the mucus network,

created when mucus is dispersed by stirring, will have some random distribution of shapes. If the individual chains forming the network of each particle are randomly arranged any expansion or contraction of the network will change the particles' volume but will not alter its shape. Thus, it seems unlikely that particles formed of the elastic network will assume a highly assymmetric shape under normal conditions and that their average shape may be reasonably approximated as spherical. If these assumptions are valid the shape factor \underline{s} of the above equation will be a constant 2.5 and should allow for estimation of the hydration of the network. This value for hydration may differ from that measured by equilibrium swelling in two respects:

1. The hydration estimated from intrinsic viscosity is an effective hydrodynamic hydration. It represents the water which travels with a network fragment as the fragment is sheared in the viscometer. This amount of water may be different from the amount of water held within the interstices of the network in an equilibrium situation.

2. The hydration of small network fragments may be different from the network as a whole. The mucus is sheared extensively in sample preparation (see below) and it is possible that this disruption changes the network properties.

In light of these factors the hydration value estimated by intrinsic viscosity may provide a better estimate than equilibrium swelling of the hydration state of the mucus as it is sheared during a pedal wave.

Tests were carried out by the following procedures. A standard curve of absorbance at 280 nm versus mucus concentration (g/ml) was constructed as follows. Two samples of pedal mucus were collected and dispersed in 10 ml of distilled water as described above for the solubility tests. Three 1 ml aliquots of each of the resulting 10 ml samples were dried at 105 °C for 24 hours and weighed to determine concentration. Other aliquots were serially diluted and their absorbance measured. Averaged values of A₂₈₀ were then plotted against concentration.

This standard curve was used to measure the concentration of mucus present in subsequent tests. For these tests samples were collected and dispersed in distilled water as described above. Samples were then centrifuged at 12,100 g for 5 minutes to remove any large particulate matter. It was shown that in the solubility tests mucus dispersed in this manner consists of particles less than 5µm in size but greater than 1µm. Samples were serially diluted and the specific viscosity measured for each concentration using an Ostwald capillary viscometer with a transit time of 80-100 seconds for water. Transit time was measured with a stop watch. Tests were repeated for one sample until the same transit time was measured three times consecutively. The viscometer was rinsed with 1 N HCl and dried between samples. All tests were carried out at 25 ± 0.1°C in a controlled temperature bath. Some of the results of these tests are presented in Figures 6.3 and 6.4 .

FIGURE 6.3. The intrinsic viscosity of the disrupted fragments of Ariolimax columbianus pedal mucus shows the characteristics of a polyelectrolyte. The intrinsic viscosity decreases as either the negative charges are masked by sodium ions, or the pH is lowered to reassociate the carboxyl groups. The two curves for distilled water represent the extremes in the range of samples tested.

FIGURE 6.3

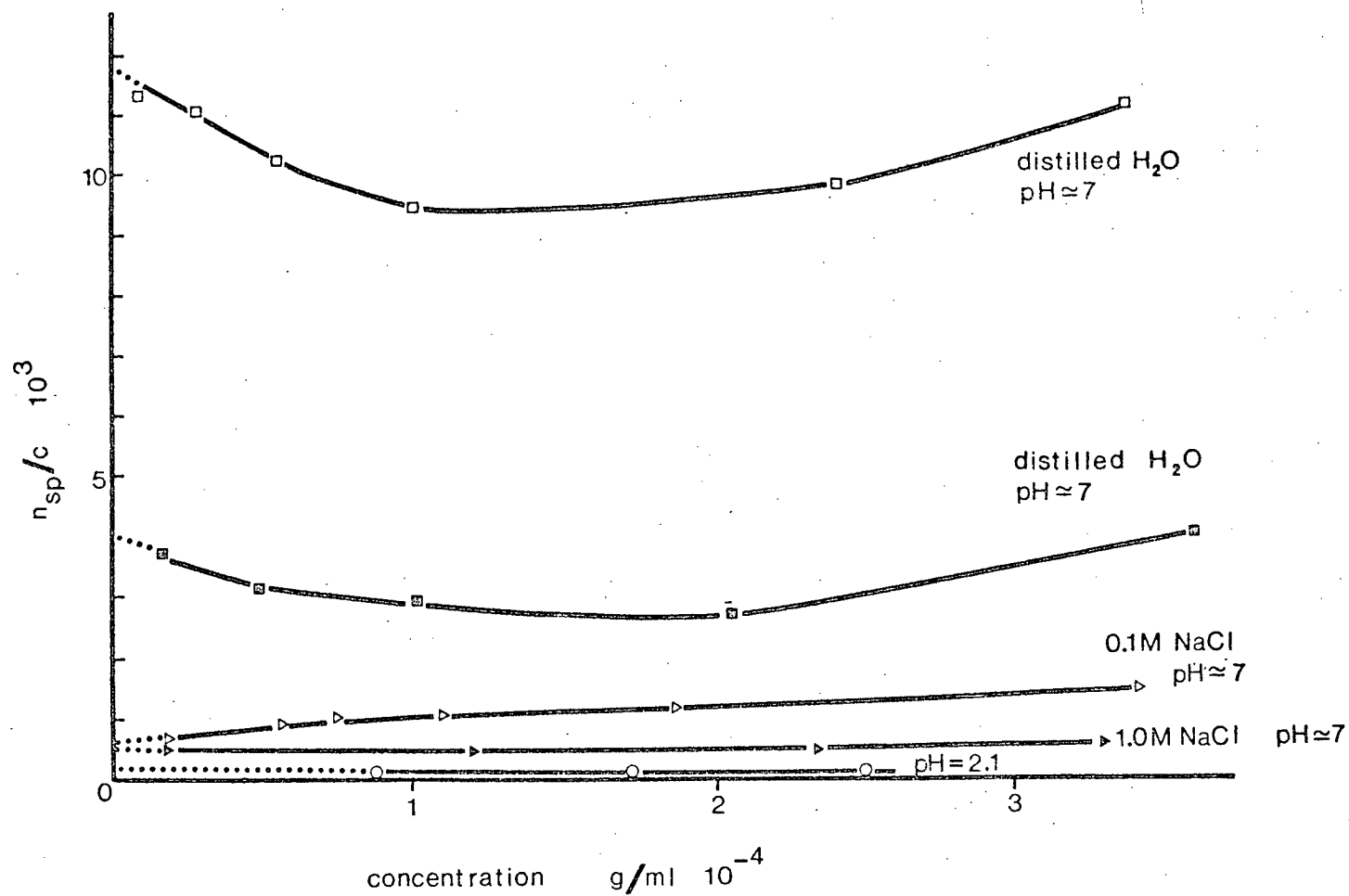
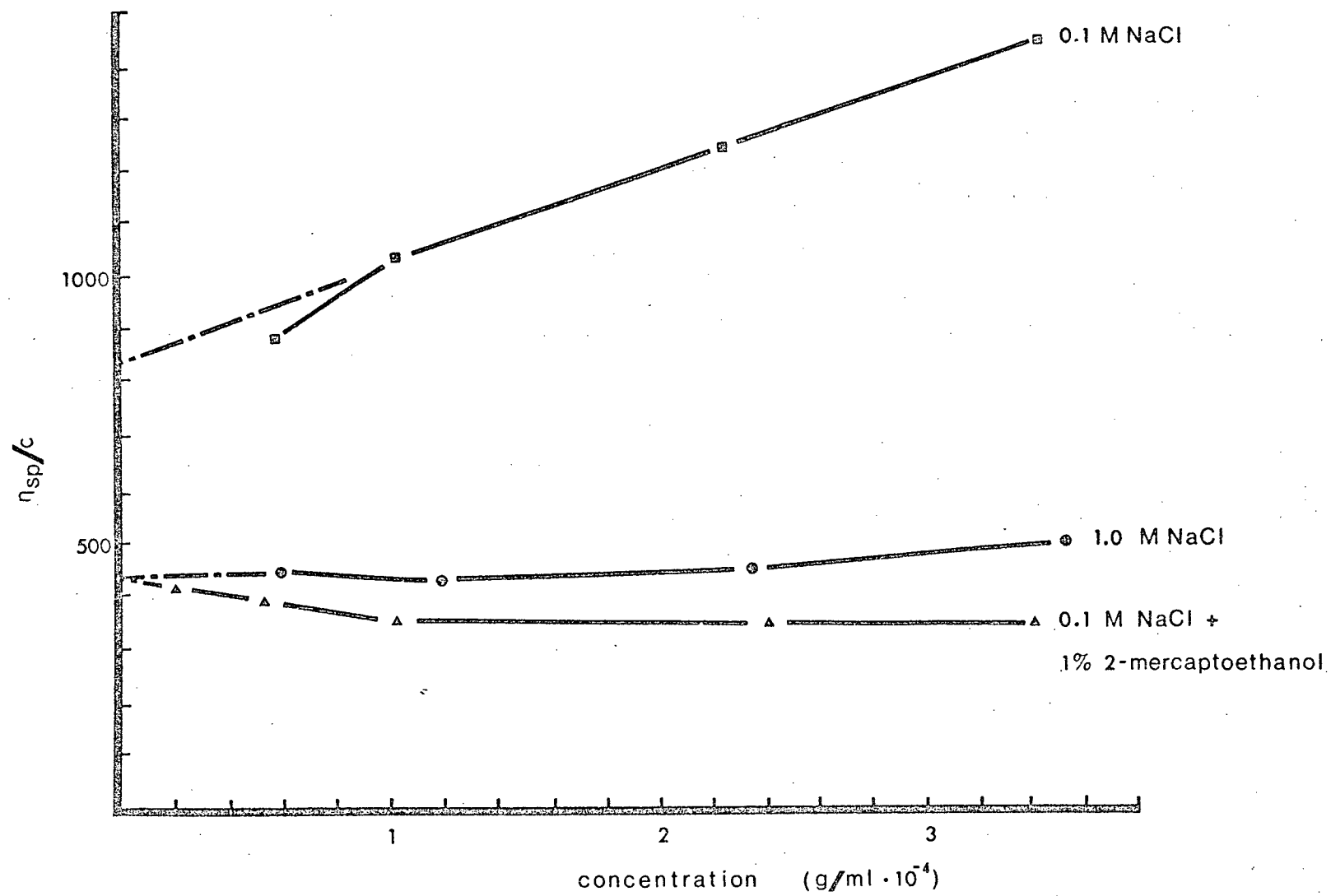


FIGURE 6.4. Molecular weight dependence of intrinsic viscosity. Dissolving Ariolimax columbianus pedal mucus with 1% 2-mercaptoethanol lowers the intrinsic viscosity observed in 0.1 M NaCl to less than or equal to that observed in 1.0 M NaCl, presumably as a result of the disruption of the mucus network.

Figure 6.4



The specific viscosities of nine samples of mucus dispersed in distilled water were measured. The results were highly variable. Values of η_{sp} ranged from 2,500 to 11,300. This variability could not definitely be attributed to any factor in the experimental procedure. The two tests that form the upper and lower boundaries of the range of tests are shown in Figure 6.3. These results are further complicated by the nonlinearity of the η_{sp}/c versus c plots. This nonlinearity makes it difficult to extrapolate the curve to zero concentration. If, however, the line formed by the two points of lowest concentrations is extended to zero and assumed to provide an estimate of intrinsic viscosity, values ranging from 4,000 to 11,500 (with an average of 6,100) are obtained. Regardless of the variation in the results of these tests all of the values of intrinsic viscosity obtained are quite large. For example they are equal to or greater than those reported for DNA, a very large and highly asymmetrical molecule. The intrinsic viscosities of several biological macromolecules are compared to that of pedal mucus in Table 6.2.

As explained above, if it is assumed that the mucus particles being tested here are rigid and roughly spherical their hydrations can be calculated. The partial specific volume of the mucus contributes negligibly, and it is assumed that the partial specific volume of the water of hydration is the same as bulk water ($=1$). Therefore the average intrinsic viscosity of 6,100 corresponds to a hydration of 2430 ml of water per gram of mucus. Even

Table 6.3: Intrinsic Viscosities of Various Macromolecules

Molecule	Molecular weight	Intrinsic viscosity
ribonuclease (globular protein)	13,683	3.3
hemoglobin	68,000	3.6
bushy stunt virus (spherical)	10,700,000	3.4
tobacco mosaic virus (cylindrical)	39,000,000	36.7
myosin	493,000	217
collagen	345,000	1150
DNA	6,000,000	5000

considering the fact that these estimates of hydration may be high due to inaccuracies in the assumptions made in the computations, it is evident that the mucus network considerably influences the water in its vicinity.

The slope of these curves is also indicative of processes occurring as the mucus is diluted in the course of testing. If the rigid particles dissolved in a fluid do not interact, the slope of the nsp/c curve should be zero (Tanford, 1961). It is usually found, however, that at finite concentrations macromolecular solutes do interact. The amount of this interaction is a function of concentration, the amount of interaction being greater at higher concentrations. Therefore a high value of nsp/c will be measured at high concentrations and nsp/c will be decreased as the molecules are diluted. This process leads to an $nsp/c/c$ curve with a positive slope. For a polyelectrolyte dissolved in distilled water this trend is counteracted by dilution of counter ions in the polyelectrolyte network. The more the sample is diluted the lower the concentrations of counterions and the more the network expands (Tanford, 1961). This expansion of the network leads to an increase in the molecular volume/molecular weight ratio and nsp/c increases as the concentration decreases. As a consequence of these two processes the nsp/c versus c plots for a polyelectrolyte goes through a minimum (Tanford, 1961). Thus the shape of the curve is further evidence of the polyelectrolyte nature of pedal mucus.

Two further viscosity tests were carried out on the whole mucus. Two samples of mucus were dispersed in 10 ml of 0.1 M NaCl. This is a salt concentration roughly equivalent to physiological concentrations in whole mucus. These samples were then tested in the Ostwald viscometer. The results of these tests are plotted in Figure 6.3. Dispersing the pedal mucus in this salt solution lowers the intrinsic viscosity by a factor of roughly 5 from the lowest value obtained in distilled water. The presence of Na⁺ counterions causes the mucus network to become more compact. Though the intrinsic viscosity is lower, its value (690) is still quite large for a biological molecule (see Table 6.2). For comparison Allen, Pain, and Snary (1976) found a value of 320 ml/g for pig gastric mucus in 0.2 M KCl. This $[\eta]$ (690, for slug pedal mucus) corresponds to a hydration of 276 ml of water per gram of mucus. One sample was dispersed in 1.0 M NaCl. This sample showed still lower intrinsic viscosity (495 ml/g) corresponding to a hydration of 197 ml H₂O/g. Neither of these samples shows the non-linear plot seen for mucus in distilled water. The presence of counterions, by decreasing the network volume and masking charges, should decrease the level of intermolecular interaction. Further the presence of a large amount of counterions in the solvent ensures a constant level of counterions in the network. Thus both processes leading to a non-linear plot are minimized and the plot becomes linear with a very small slope.

Two samples were dispersed in distilled water as for

previous tests and then the pH of the samples was adjusted to pH 2.1 using HCl. The experimental points from these tests are also plotted in Figure 6.3. Lowering the pH well below the pK of the network's carboxyl groups lowers the intrinsic viscosity even further than does the presence of counterions. Presumably in its non-charged state the polymer network of the mucus assumes a random configuration and the volume of this configuration is less than that of a charged network containing counterions. The intrinsic viscosity of mucus particles under these conditions is 60 to 70 ml/g, corresponding to a hydration of 24 to 28 ml H₂O/g. In Table 6.1 the hydration values estimated here from intrinsic viscosity measurements are compared to the values obtained from equilibrium swelling. For the NaCl solutions the two figures match fairly closely. The two values may be matched exactly if the shape factor, s , of equation 6.1 is changed from 2.50 to 3.47. The value of 3.47 corresponds to an ellipsoid particle with a major axis/minor axis ratio of between 2.7 and 3.0 (depending on whether the ellipsoid is prolate or oblate, respectively). Thus these data are consistent with the possibility that the network fragments are slightly elongated, hydrodynamically rigid particles in these salt solutions.

At the extremes of the swelling range the estimates of hydrodynamic hydration diverge considerably from equilibrium hydration, being larger in distilled water and smaller at pH 2.1. It may be speculated that the presence of a large number of charges in distilled water and the near total lack

of charges at pH 2.1 somehow affects the amount of water bound to the moving particle. This could account for this discrepancy. However a mechanism for this effect is not immediately evident and the matter will require further study. Regardless of the precise comparison of hydrodynamic with equilibrium hydration these tests reconfirm the fact that the mucous network influences very large amounts of water.

All of the above tests were made on mucus similarly prepared. Consequently differences in $[\eta]$ are attributable to either a change in hydration, shape, or both. If, however, the mucus is dissolved by treatment with 2-mercaptoethanol many of the crosslinks of the network are broken and the molecular weight of the resultant particles should be less than that of the network fragments of untreated mucus. It seems reasonable to assume that much of the water which would be trapped in the interstices of a gel network will be "released" (ie. not hydrodynamically bound) when the network is dissolved. In other words, glycoprotein chains can affect more water when they are crosslinked together (by enclosing spaces like a sponge) than they can separately. Thus, as mercaptoethanol dissolves the network the intrinsic viscosity should decrease. This is indeed the case. Figure 6.4 shows the results from a comparison of untreated mucus in 0.1 M NaCl with mucus that has been treated for 24 hours at 40 °C with 2-mercaptoethanol. Both tests were performed at 25 °C. As predicted the intrinsic viscosity of the dissolved mucus is

considerably lowered, further confirming the fact that 2-mercaptoethanol effectively dissolves the mucous network.

In summary, these measurements of the intrinsic viscosity of pedal mucus show that:

1. Even under non-equilibrium conditions the mucous network influences large volumes of water.
2. The fact is reconfirmed that the mucous network expands and contracts depending on the nature of the solvent; evidence of the polyanionic nature of the glycoprotein.
3. 2-mercaptoethanol is shown to reduce the molecular weight of the mucous particles in solution.

Molecular Weight Between Crosslinks

One of the predictions of the theory of rubber elasticity is that the modulus of an entropy elastic material should be related to the number of crosslinks present per volume of material. If very few crosslinks are present, the majority of chains in the network will be free to rearrange randomly as the network is stressed and the network's stiffness will be low. The more crosslinks present, the less freedom chains will have when the network is deformed and the stiffer the network will be. Now the more crosslinks there are in a given volume of network the shorter the lengths of chain between crosslinks. Since each length of chain will contain a certain weight of polymer molecules a relationship should exist between the molecular weight between crosslinks (and thereby chain length and

number of crosslinks) and the modulus of the material.

Theory states that this relationship is of the form:

$$G = pRT/M$$

where p is the density of polymer chains in the network (in g/ml of gel), R is the gas constant (8,300 ergs/degree mole), T is absolute temperature, and M is molecular weight between crosslinks. In order for this equation to be strictly true, the value of G used must be the equilibrium value. This is the value of G obtained from a stress relaxation test at infinite time. In this manner the equation is a measure of the molecular weight between permanent crosslinks. Stress relaxation tests on A. columbianus pedal mucus (Chapter 4) show that this material does not have an equilibrium modulus and therefore this equation in its strictest sense cannot be applied. However over short periods of time, pedal mucus does have a modulus, presumably as the result of temporary crosslinks. Thus, if this instantaneous modulus is used, the molecular weight between temporary crosslinks can be estimated. The equation is applied in the following manner as suggested by Alexander (1965) and Ferry (1970). The value for G is taken from the tests of Chapter 4 to be 100 to 200 N/m². These measurements were conducted at about 20 °C (293 °K). The density figure used is that of the mucous network (not including the water with which it is mixed) or about 0.03 g/ml. Using these values the figures for molecular weight

between crosslinks can be calculated as 8.8 to 7.5×10^5 . This represents the minimum molecular weight that glycoprotein chains could be and still form the elastic network found in mucus. If more than one crosslink is present on each glycoprotein molecule, the molecular weight of the whole molecule will be some multiple of this weight between crosslinks.

Gel Filtration

The composition of solubilized mucus was examined by gel filtration. This technique uses the properties of an agarose gel to separate molecules on the basis of size. Large molecules applied to such a gel column are too bulky to fit into the interstices of the column's gel beads (they are "excluded"). These large molecules are washed between the gel beads and are rapidly eluted. For a Sepharose 4B column, as used in this study, compact molecules with a molecular weight of greater than 20 million are excluded. However, the more expanded glycoprotein molecules are excluded at a lower molecular weight. Snary, Allen, and Pain (1970) found that pig gastric mucus glycoproteins with a molecular weight of 5.5 million or larger were excluded on Sepharose 4B.

A column of Sepharose 4B (CL) (100cm by 2 cm) was used for the separation. The column was equilibrated to a solvent of 0.1M NaCl containing 1% 2-mercaptoethanol. Mucus samples were collected and dispersed in 1% 2-mercaptoethanol and allowed to solubilize at 40 °C for 24 hours. Samples

were then centrifuged for 5 minutes at 12,100 g to remove particulate matter. A 10 ml aliquot of this solubilized sample was loaded on the column and eluted with 0.1M NaCl, 1% 2-mercaptoethanol at a flow rate of about 12 ml/hour. All tests were conducted at room temperature 21 to 23 °C. Ten ml fractions were collected and assayed for carbohydrate and protein. Absorbance at 280 nm was used to measure protein concentration. The phenol - sulfuric acid assay of Lo, Russel, and Taylor (1970) was used to measure carbohydrate concentrations. The procedure was repeated several times with similar results. The composite chromatogram of three of these tests is presented in Figure 6.5.

This test indicates that at least two major fractions of molecules are released when the disulfide crosslinks of the mucus are broken by 2-mercaptoethanol.

1. A larger molecule containing carbohydrate and probably a small amount of protein.

2. A smaller molecule containing only protein.

In two tests a third protein peak was found eluting before the carbohydrate peak. In these cases the main protein peak (peak 2; see Figure 6.5) was smaller. It is assumed that this type of elution profile is accounted for by the incomplete solubilization of the crosslinked network leading to the presence in the sample of both uncrosslinked protein chains (peak 2) and large complexes of crosslinked protein networks. An example of one such chromatogram is shown in Figure 6.6.

FIGURE 6.5. Separation of pedal mucus on Sepharose 4-B CL results in the elution of two major fractions: 1) a high molecular weight fraction containing both protein and carbohydrate, and 2) a lower molecular weight fraction containing protein alone.

FIGURE 6.5

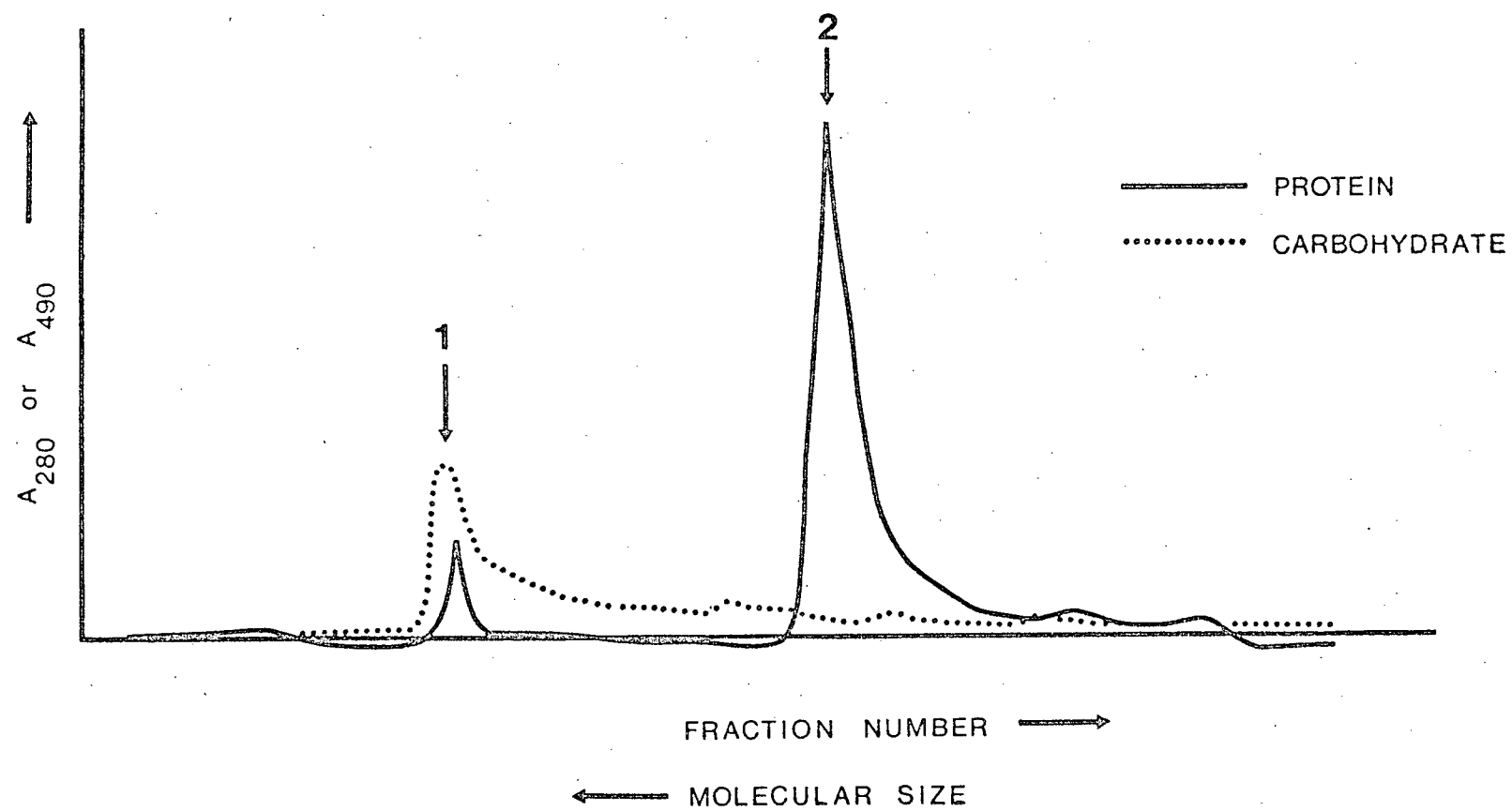
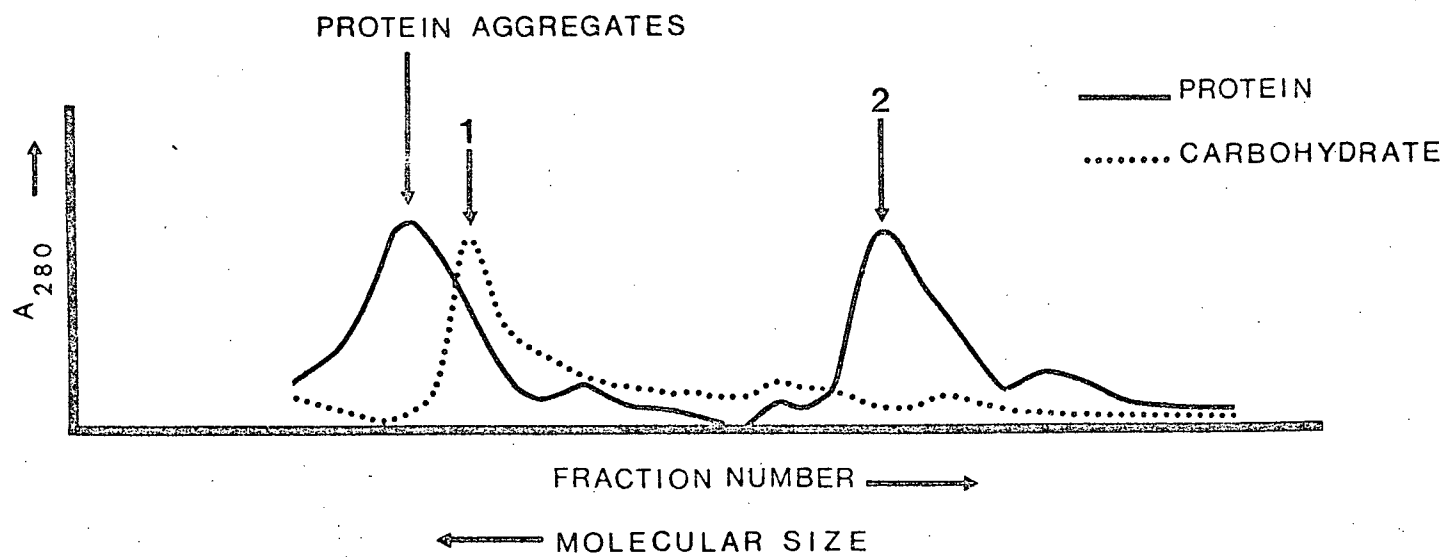


FIGURE 6.6. Incompletely dissolved pedal mucus shows a third fraction when separated on Sepharose 4-B CL. This is presumably due to aggregates of the protein of fraction 2.

Figure 6.6



Attachment Of Carbohydrate To Protein

It has been shown (Gottschalk, 1972; Hunt, 1970) that for many glycoproteins the polysaccharide chains are bound to the protein through O-glycosidic bonds to either serine or threonine. The presence of such bonds may be easily detected (Carubelli *et al.*, 1965). The O-glycosidic bond of a hexose to a serine or threonine is unusually labile in weak alkali solutions, and is broken through a process known as B-elimination. When the bond is cleaved the amino acid is converted to a compound that absorbs strongly in the ultraviolet. Normal glycosidic bonds and peptide bonds are not affected by treatment with mild alkali. Thus, the reaction between a dilute solution of NaOH and a glycoprotein may be carried out in a spectrophotometer and an increase in absorption at 241 nm is indicative of the presence of O-glycosidic bonds between hexose and serine and/or threonine.

A. columbianus pedal mucus was collected and dispersed in distilled water as for the solubility tests described above. One milliliter of this mucus solution was mixed with one ml of distilled water and served as an absorbance standard. Another milliliter of mucus solution was thoroughly mixed with 1 ml of 1 N NaOH for a final concentration of 0.5 N NaOH. The absorbance of this sample (at 241 nm) was read relative to the standard in a Pye Unicam 1750 dual beam ultraviolet spectrophotometer. The tests were conducted at room temperature. Absorbance values were recorded for 15-20 minutes after the initial mixing.

The experiment was repeated with a final sample concentration of 0.25 N NaOH. A mucous solution mixed with an equal volume of distilled water served as a control. All tests were performed in triplicate and the results are presented in Figure 6.7.

Mucus samples treated with NaOH show an increasing absorbance at 241 nm. The rate of increase of absorbance decreases after about ten minutes. If the rate of increase is computed for the initial ten minutes of the experiment the value obtained with glycoprotein in 0.50 N NaOH is almost exactly twice that obtained with 0.25 NaOH. The absorbance of the control did not change with time.

These results are very similar to those of Carubelli et al (1965) and are taken as evidence (though not conclusive) that the polysaccharide component of A. columbianus pedal mucus is at least in part connected to the protein component by O-glycosidic bonds to serine and/or threonine. Further treatment of the alkali treated mucus and amino acid analysis of the resulting compounds should provide additional evidence concerning this point.

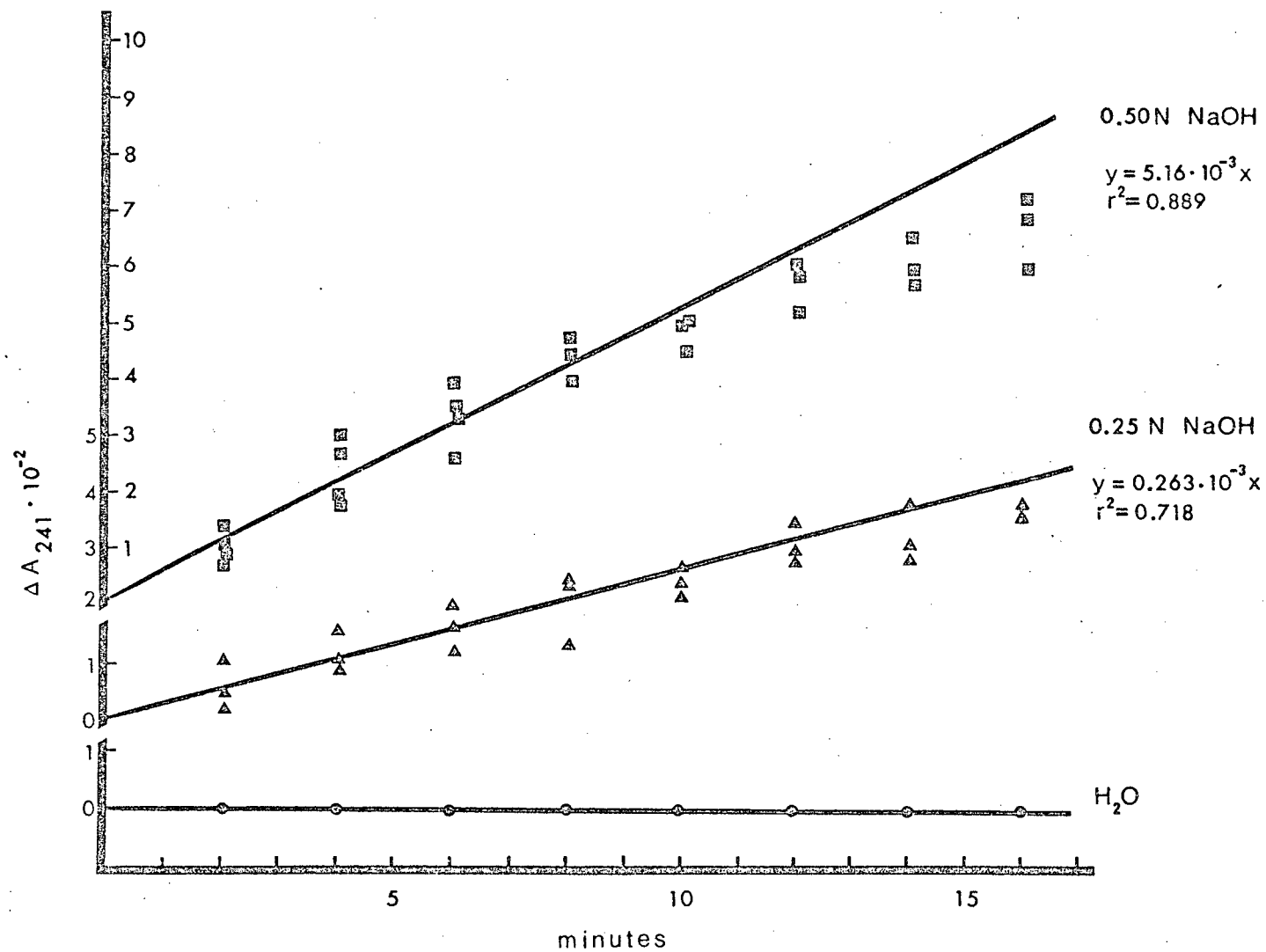
Summary

In summary, the results of these tests indicate that:

1. The elastic network of A. columbianus pedal mucus is formed from a polyanion.
2. The molecular weight of this polyanion is quite large (greater than 7.5×10^5).
3. At least two sorts of molecules contribute to the

FIGURE 6.7. Carbohydrate bonding to serine and/or threonine. The increase in absorbance at 241 nm. of mucus in weak NaOH solutions indicates that polysaccharide is covalently bound to protein in Ariolimax columbianus pedal mucus.

Figure 6.7



mucus network, a large molecule consisting primarily of polysaccharides and a smaller molecule composed of protein.

4. The carbohydrate portion if the glycoprotein is apparently covalently bonded to the protein, the attachment site(s) being serine and/or threonine.

5. The mucus network is crosslinked both by disulfide bridges between protein molecules, and weak bonds between either the carbohydrate portion, the protein portion, or both.

6. The crosslinked, highly expanded mucous network influences large amounts of water.

These tests form only a very cursory study of the physical chemistry of A. columbianus pedal mucus. Many questions remain to be answered.

Comparison With Other Mucins

A considerable amount of study has been directed at the physical chemistry of mucous secretions. As with other studies on mucins these studies have generally been limited to the study of vertebrate (primarily mammalian) glycoproteins and mucopolysaccharides. The findings of these studies are reviewed in Gottschalk (1972), Hunt (1970), Elstein and parke (1976), and the British Medical Journal (1978).

Recently a number of authors have made sufficient progress in examining the physical chemistry of a variety of mucins to be able to propose models for mucus structure (Allen and Snary, 1971; Allen, 1978; Roberts, 1978; Rao and

Massen, 1977). All of these models incorporate three similar features.

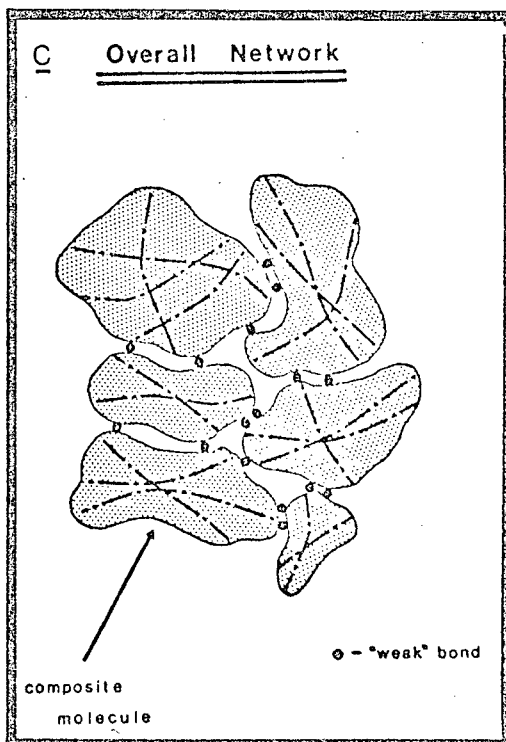
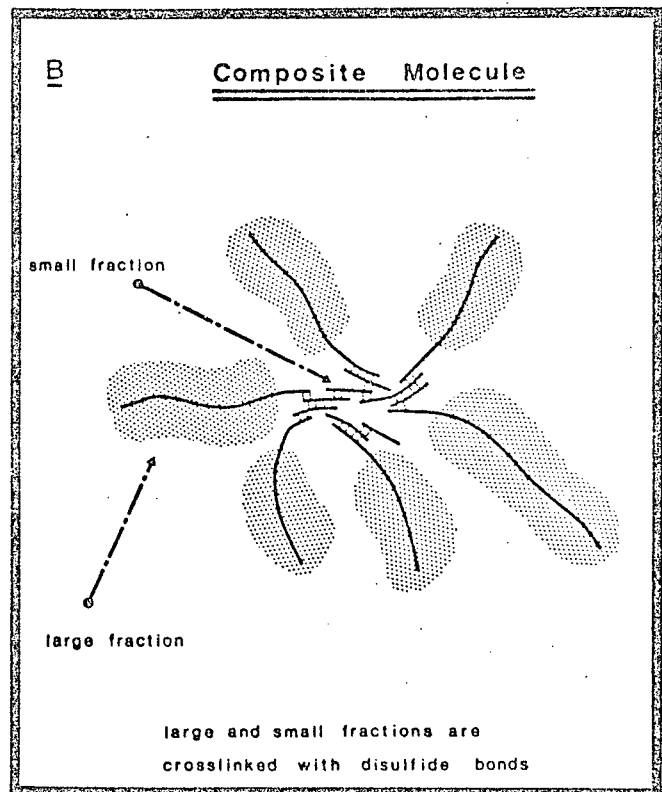
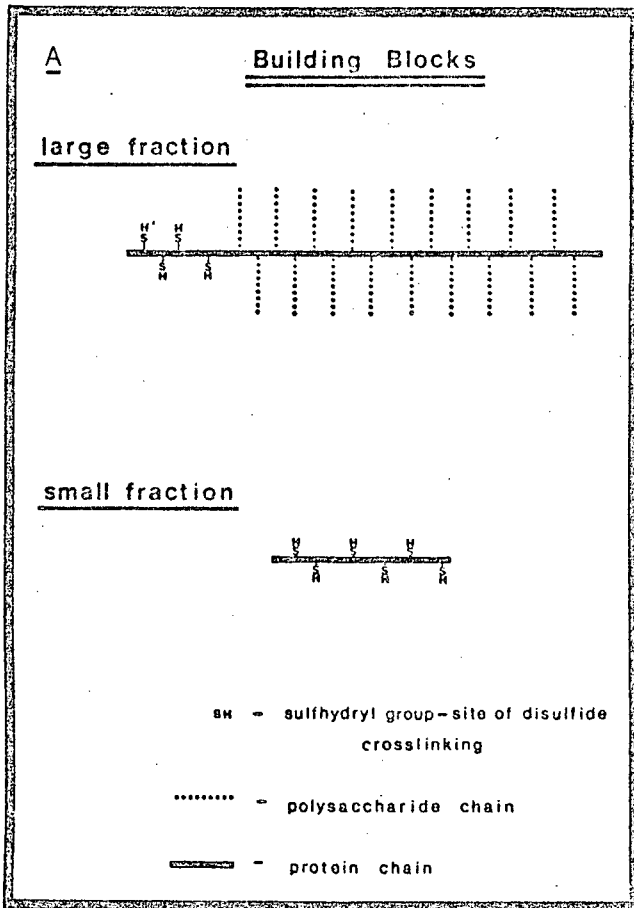
1. Subunits consisting of a protein core with carbohydrate side chains.
2. "naked" areas on the protein core where disulfide bonds can be formed between two subunits.
3. Some form of interaction (hydrophobic or other "weak" bonds) between large composite molecules formed of a number of crosslinked subunits.

Thus several subunits are crosslinked (S-S) to form larger molecules. These molecules in turn interact to form the mucus gel. It seems likely that this basic sort of model is valid for a large number of vertebrate mucins. It is interesting to speculate how an invertebrate mucin (such as A. columbianus pedal mucus) might compare.

There appears to be only one minor problem in reconciling the scant data presented in this study with this vertebrate model. A. columbianus pedal mucus appears to contain a large portion of protein that is not covalently bound to carbohydrate. This fact may easily be incorporated into a model as shown in Figure 6.8. Small lengths of protein are bound together by disulfide bonds. Some of these small proteins are in turn linked to the protein core of the protein/polysaccharide chain. In this manner large composite molecules of glycoprotein are constructed. These large molecules then interact through weak bonds and entanglements to form the overall mucus network. When the mucus is sheared under the foot of the slug, the weak bonds

FIGURE 6.8. A model for the structure of Ariolimax columbianus pedal mucus. The two fractions observed in Figure 6.5 are crosslinked by disulfide bonds to form large composite molecules. These composites subsequently interact by "weak bonds" to form the overall mucus network.

FIGURE 6.8



between large composite molecules are ruptured and the mucus flows. When shearing is terminated the weak bonds rapidly reform, and the mucous network "heals". The longer the time allowed for healing, the more weak bonds and entanglements are formed and the higher the modulus of the network.

At present this model for A. columbianus pedal mucus structure is pure speculation. A considerable amount of work must yet be performed before this model can be substantiated or discounted. It will be interesting to follow the course of future research to see if this type of model is indeed applicable to slug mucus and to invertebrate mucus in general. The presence of a simple, general model for mucus structure would be extremely useful as a basis for further research into the structure and function of mucous secretions.

At present I can propose no model for the mechanism of fiber formation in A. columbianus pedal mucus. Again, further research is required.

CHAPTER SEVEN

A Model For Slug Locomotion

Complex biological problems, such as gastropod locomotion, are often best examined through the use of a model. A model in this sense is a hypothetical structure used to relate isolated facts known about a process and thereby provide predictions about that process. If on the basis of known facts a model provides correct predictions, the hypothetical structure of the model can be thought of as corresponding to the actual structure.

The qualitative model proposed and tested by Lissman (1945b) has been, to date, the only model to examine the mechanism of gastropod locomotion. This model is based on the kinematics of snails of the genus Helix (Lissman, 1945a). As the locomotory movements of Helix are very similar to those of A. columbianus (described in Chapter 3), Lissman's model should be applicable to the problem of slug locomotion.

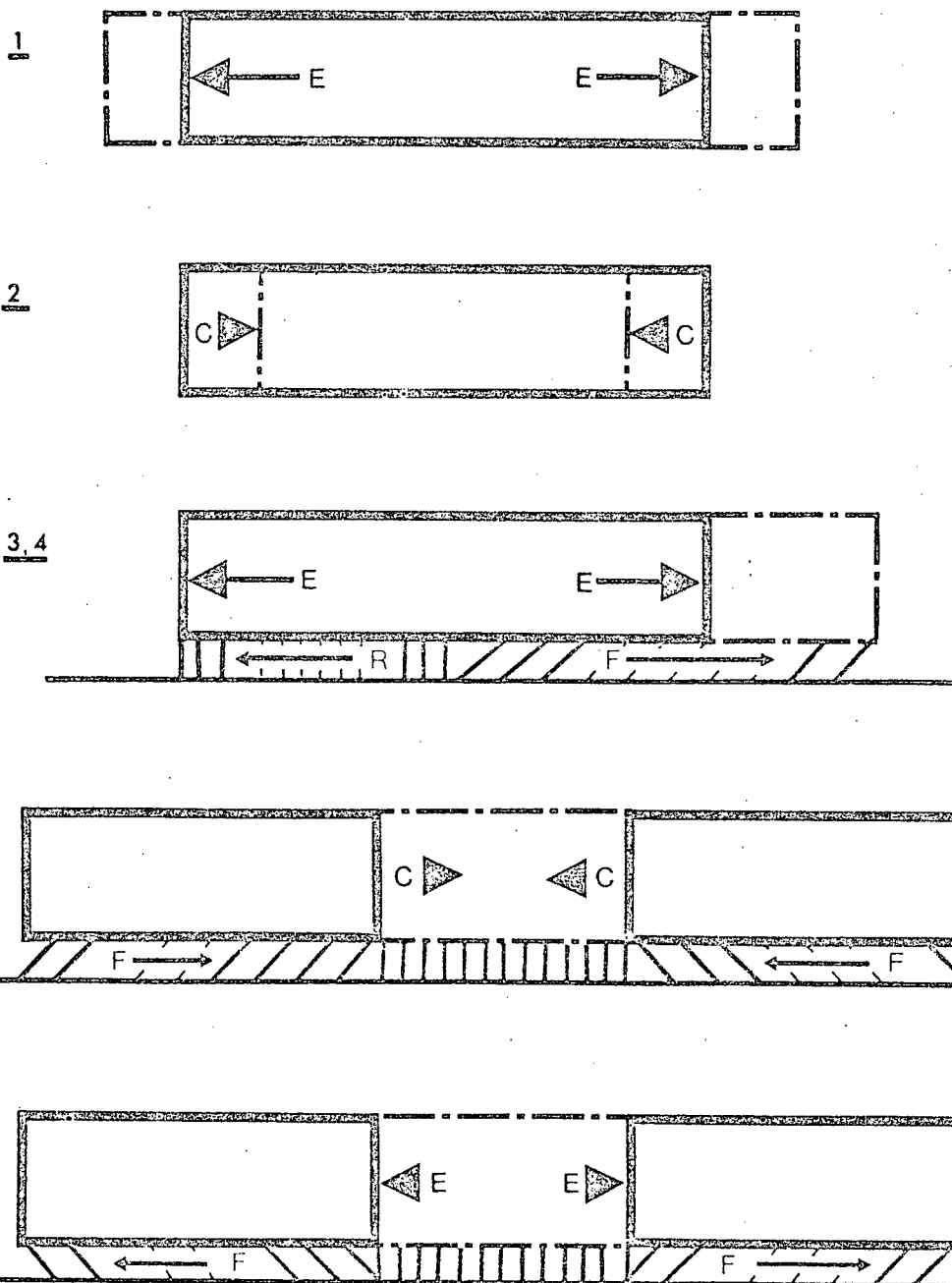
Lissman proposes that the locomotion of snails (or slugs) can be accounted for by the interaction of four and possibly five, types of forces as shown in Figure 7.1:

1. Forces acting to extend the foot (most probably hydrostatic pressure) ;
2. Forces acting to compress the foot (muscular contraction) ;
3. A frictional drag between the substratum and the segments of the foot that are moving forwards (a function of

FIGURE 7.1. The possible forces acting during gastropod locomotion (after Lissman, 1945b).

- 1) An internal force of expansion causes the foot to elongate.
- 2) An internal force of compression causes the foot to become shorter.
- 3) and 4) An elongating foot segment in contact with the ground will result in a frictional resistance to movement, F , and a reactive force resisting this movement, R .
- 5a and b) Compression or elongation of the central of three segments will result in a frictional resistive force.

Figure 7.1



the properties of pedal mucus) ;

4. A reactive force opposing the frictional drag.

This force also acts through the mucus under those segments of the foot which are stationary (the interwave) ;

5. Possibly tensions or thrusts between stationary segments of the foot.

Of these five forces, 3, 4, and 5 can be quantified by measuring the forces exerted on the substratum by a moving slug. Lissman attempted to measure these forces and in his analysis relates them to produce a qualitative model for the locomotion of Helix .

This model suffers from several problems: First it is strictly qualitative. Without knowledge of the properties of pedal mucus, predictions of the magnitude of the frictional drag and resistive forces was impossible. While Lissman's model is in most respects qualitatively accurate, it is much more difficult to critically evaluate such a model than a model where both the direction and magnitude of forces can be compared to reality.

Second, the force transducer used by Lissman may well have been inappropriate. The apparatus consists of a platform mounted on a pendulum, the more force placed on the platform, the larger the displacement of the pendulum. The precise dimensions of the apparatus are not given, but from diagrams shown in Gray and Lissman (1938) it can be estimated that the period of the pendulum is about 0.5 to 1.0 seconds. If this is so, the device is incapable of accurately measuring oscillating forces with a period of

around one second or less. The forces measured by Lissman fall squarely into this category.

In order to explain what are probably inaccurate force measurements, Lissman complicates a basically simple model by invoking tensions and thrusts between stationary segments of the foot (type 5 force of Figure 7.1) . These forces lead him to argue that "the propulsion of the front end of the animal is effected by regions of the foot lying posterior to itself, whereas the hind end is being aided by a pull from regions lying more anteriorly ". This sounds suspiciously like someone trying to lift himself up by his bootstraps, and causes considerable confusion in interpreting Lissman's model.

In light of the facts presented in Chapters 1-6 of this thesis, a quantitative model can be constructed which incorporates many of the features of Lissman's model but avoids the problems. This chapter will present this model, its predictions, and tests of these predictions.

The Model

Before examining the precise calculations which form the model, it will be useful to outline the model's major points and show how these differ from Lissman's.

The present model is designed to predict the forces operating under a slug crawling on a smooth nonporous, inflexible surface. Under these conditions, as shown in Chapter 3, the slug does not lift the foot during locomotion. Thus, as the slug moves along it passes over a

layer of mucus of a constant thickness. Various stresses are imposed on this mucus layer by the moving foot. The magnitude of these stresses can be estimated from a knowledge of the movements of the foot and the physical properties of the pedal mucus.

As shown earlier the foot may be divided into three functional areas: the waves, the interwaves, and the rims. The stresses associated with each area will be examined in turn. The stresses are diagrammed in Figure 7.2. This model calculates the stress associated with each area and relates these stresses to account for locomotion.

The Waves

As a segment of the foot is overtaken by a compressional pedal wave, it begins to move forward. This movement will shear the mucus beneath that segment. The shear ratio, ρ , will be equal to x/y where x is the distance moved by the foot and y is the mucus layer thickness (see Figure 7.2). At some point this mucus will be sheared beyond its yield point and it will flow. Subsequently, as long as the segment of the foot is moving forward it will be moving over a viscous fluid. The viscosity of the fluid and the shear rate imposed by the moving segment will determine F_w , the resistance to movement experienced by the moving wave:

$$\text{shear stress, } 2 \text{ gma} = n \, dp/dt$$

$$F_w = \sigma A_w$$

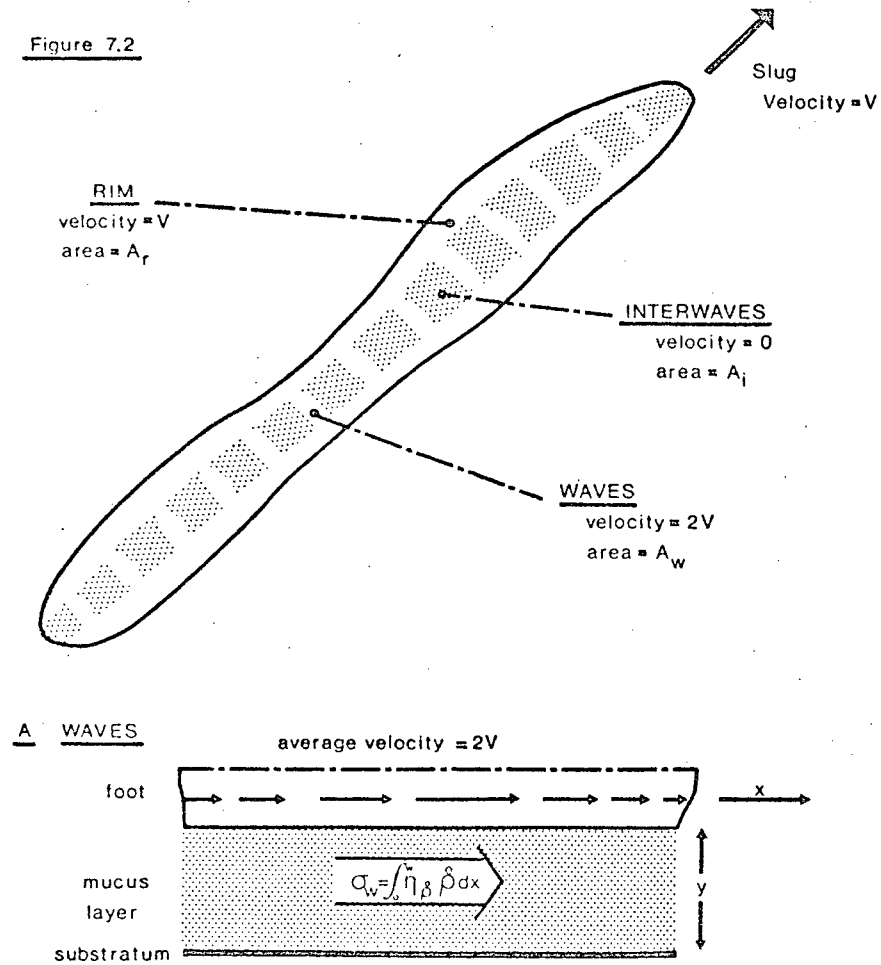
FIGURE 7.2. The forces present under a moving slug.

- A) Waves. The stress at any point is equal to the shear rate times the viscosity at that shear rate. The sum of all stresses under a wave is the wave stress. The wave stress times the wave area equals the wave force, F_w .
- B) Rim. The rim stress is equal to the shear rate under the rim times the viscosity at that shear rate. The rim stress times the rim area equals the rim force, F_r .
- C) Interwaves. In order for locomotion to occur

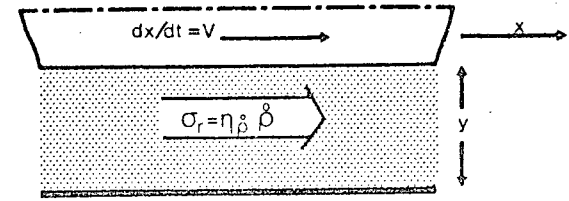
$$F_i = -(F_r + F_w)$$

where F_i is the interwave force. Thus the interwave stress equals F_i divided by the interwave area.

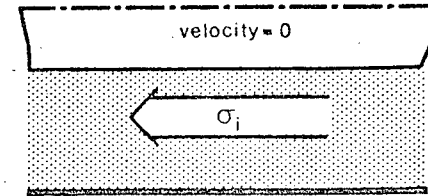
Figure 7.2



B RIM



C INTERWAVES



where n is the value of viscosity from Figure 7.4, and A_w is the area of the foot contained in the waves. As shown in Figure 7.1 this resistance will place an equal and opposite force on the stationary portions of the foot - the interwaves.

The Rims

The rims of the foot move forward at the same constant speed as the slug itself. Consequently, once sheared beyond its yield point when the slug begins to move the mucus beneath these rims will always remain in its fluid form as long as the slug continues to move. Again the rate of movement of the rim and the viscosity of the fluid will determine F_r , the resistance to movement.

$$\text{Sigma} = n \, dp/dt$$

$$F_r = \text{sigma} \, A_r$$

where A_r is the area of the rims. The force required to move the rims forward will place an additional stress on the mucus beneath the interwaves.

The Interwaves

As a wave leaves a segment of the foot behind, the segment will decelerate until it is stationary. As soon as the segment is stationary the mucus beneath it will begin to heal, becoming more solid as time passes. If the mucus heals quickly enough most of the stationary segment will

rest on mucus in its solid form. This solid mucus will hold the interwave stationary against the force of the waves and interwaves moving forward. The magnitude of the stress on this solid mucus is the force of forward motion $(F_r + F_w) / (A_i)$ the area of the interwaves. As long as the solid mucus is sufficiently strong to resist the stress imposed by the rims and waves, the slug will be able to crawl.

This model does not allow for the presence of tensions or thrusts between interwaves (type 5 force of Lissman). This is simply another way of saying that the length by which the foot contracts on entering a pedal wave is simultaneously offset by the foot re-extending on leaving a wave. This assumption considerably simplifies the model and, it will be shown, does not detract from its accuracy. Similarly this model does not take into account forces due to the acceleration or deceleration of segments of the foot. This simplification is justified by the fact that the masses and rates of acceleration are negligibly small. Further, for each part of the foot that is accelerating there is an equivalent segment decelerating so that the small forces present should tend to cancel. Finally, this model does not account for the action of those cilia present on the pedal epithelium.

With this general scheme in mind a specific example may be examined: This example makes use of an "average" slug as determined by the kinematic studies of Chapter 3. An average slug weighs approximately fifteen grams and has a total foot area of 15 cm². Slightly more than a third (5.5

cm²) is contained in the rims. Of the remaining 9.5 cm², 6.0 cm² is in the interwaves, and 3.5 cm² in waves.

Now let this slug move forward with pedal waves resembling the average wave shown in Figure 7.3 (reproduced here from Figure 3.4) . The slug (and consequently the rims) moves with a constant speed of 0.85 mm/sec. The waves, because they only move for half the time move at an average speed twice that of the slug, or 1.7 mm/second . However at any given time the precise speed of a segment in the wave will vary as shown in Figure 7.3 . The interwaves are stationary. For these foot speeds the shear rate experienced by the pedal mucus will be determined by the thickness of the mucus layer. In order to examine the effect of mucus layer thickness, stress values will be calculated for two thicknesses, 10 μ m and 20 μ m, corresponding to the approximate limits of the range of thicknesses observed in histological sections. As a convenience, only the 10 μ m example will be discussed in the text, however, all values will be found in the summary presented in Table 7.1. Given these specific values for the dimensions of the mucus layer and the movement of the foot, the stress under each of the sections of the foot can be calculated.

Waves

Under a wave the mucus is first stressed to its yield point, and then flows. Take for example a 10 μ m mucus layer thickness. This layer will yield when the shear ratio is

FIGURE 7.3. The stress profile beneath a pedal wave. The stress has been calculated for the velocity profile of Figure 3.4 for mucus layer thicknesses of 10 and 20 μm .

Figure 7.3

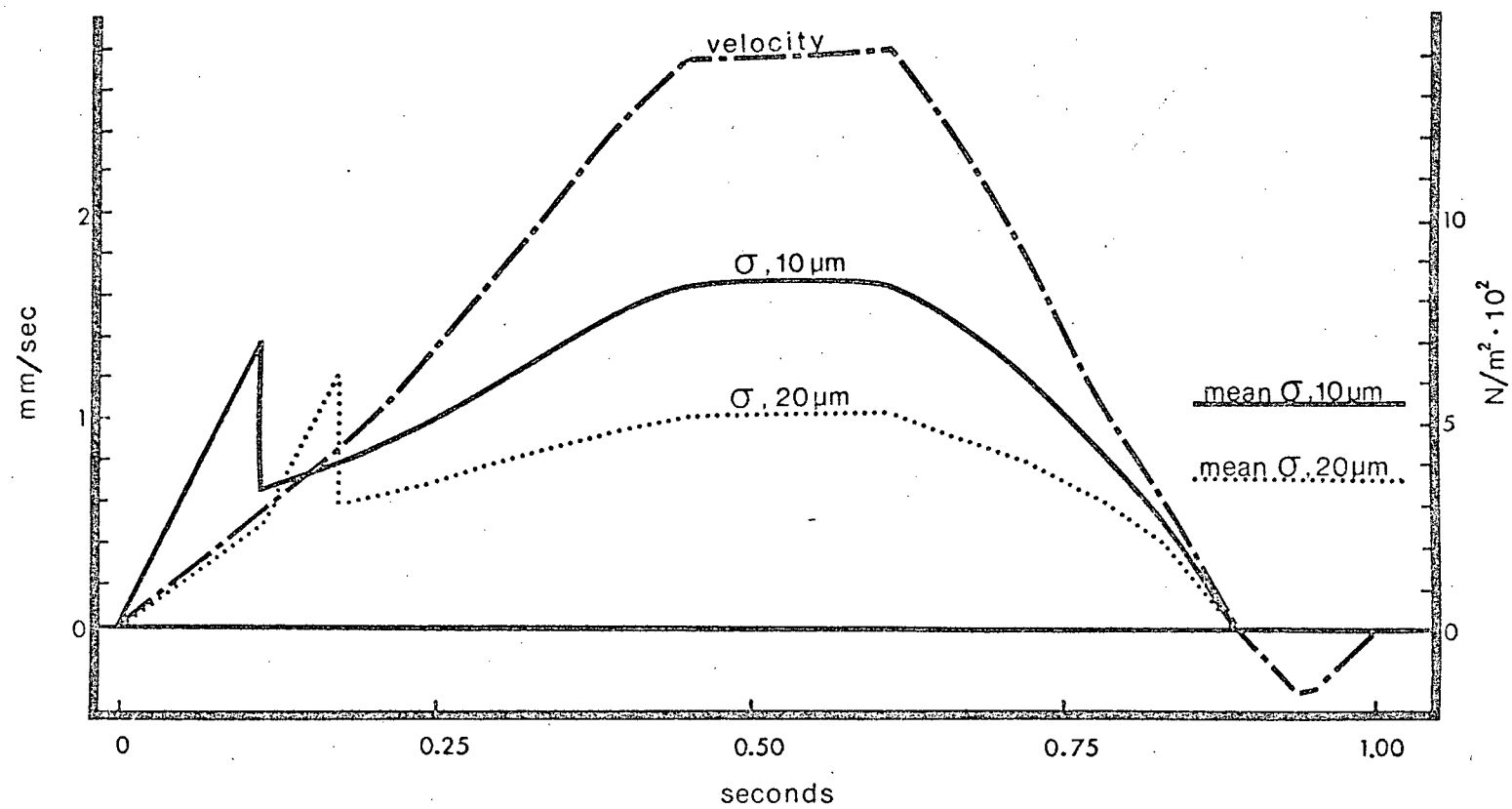


Table 7.1: Predictions of the Locomotion Model

Slug: weight.....15 gm				
foot area.....rims		$5.5 \times 10^{-4} \text{ m}^2$		
waves		$3.5 \times 10^{-4} \text{ m}^2$		
interwaves		$6.0 \times 10^{-4} \text{ m}^2$		
mucus thickness	<u>0.85 mm/sec.</u>		<u>2.00 mm/sec.</u>	
	10 m	20 m	10 m	20 m
shear rate (rims)	85/sec	42.5/sec	200/sec	100/sec
shear stress (rims)	395 N/m^2	297 N/m^2	659 N/m^2	429 N/m^2
force (rims)	0.217 N	0.163 N	0.363 N	0.236 N
average shear stress (waves)	542 N/m^2	365 N/m^2	1059 N/m^2	610 N/m^2
force (waves)	0.193 N	0.127 N	0.371 N	0.213 N
total force (waves+rims)	0.410 N	0.290 N	0.724 N	0.449 N
shear stress (interwaves) (horizontal)	682 N/m^2	485 N/m^2	1222 N/m^2	749 N/m^2
overall stress amplitude (waves+interwaves) (horizontal)	1232 N/m^2	850 N/m^2	2281 N/m^2	1359 N/m^2
overall stress amplitude (vertical up)	1482 N/m^2	1100 N/m^2	2531 N/m^2	1609 N/m^2
overall stress amplitude (vertical down)	970 N/m^2	600 N/m^2	2031 N/m^2	1109 N/m^2

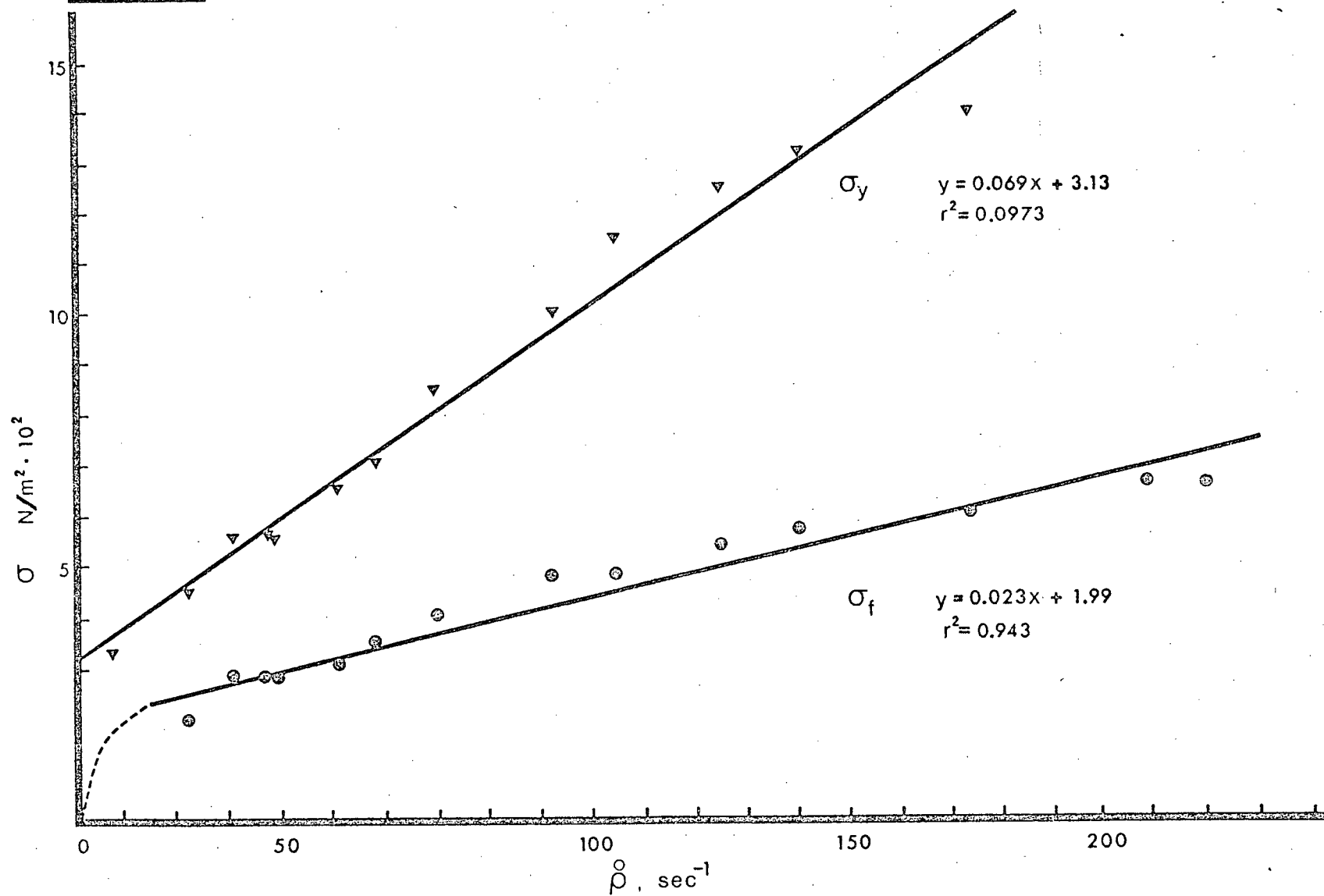
5.5; ie at a foot displacement of 55 μm . Noting in Figure 7.3 the point on the distance curve equal to 55 μm the corresponding point on the velocity curve can be found. This is equal to 0.54 mm/second or a shear rate of 53/second (see Figure 7.4). By referring to Figure 7.4 (reproduced from Figure 4.16) the stress at which a typical mucus sample yields at a shear rate of 54 can be determined (685 N/m^2). The stress to which this sample yields can also be determined; it is the value for flow stress at shear rate equal to 54/sec, or 323 N/m^2 . As the foot continues to move forward in the pedal wave the stress can be similarly calculated for every part under the moving segment. This force profile, corresponds to the average velocity profile as shown in Figure 7.3. By integrating the area under this force/time curve and then dividing by time the average stress under a wave can be calculated. For the case of a 10 μm thick mucus layer this value is 550 N/m^2 . Similarly the force profile can be calculated for a 20 μm thick mucus layer, and this is also shown in Figure 7.3. Since a thicker layer will result in a lower shear rate, the forces involved in moving over this thicker layer are smaller. This stress can then be multiplied by the total area of the waves ($3.5 \cdot 10^{-4} \text{m}^2$) to arrive at a figure for the total force necessary to move foot segments forward with pedal waves. This value is 0.19 N for a 10 μm mucus layer.

Rims

The rim force is more simply calculated. Since the

FIGURE 7.4. A representative plot of yield stress and flow stress versus shear rate for Ariolimax columbianus pedal mucus.

Figure 7.4



rims move at a constant 0.85 mm/second, the shear rate beneath them is 85/second for a 10 μm layer (Figure 7.2) . The flow stress for this shear rate is determined by reference to Figure 7.4 (395 N/m² for a 10 μm layer) . This stress value multiplied by the total rim area yields the force needed to move the rims at this speed. This value is 0.22 N for a 10 μm mucus layer.

The Interwaves

The force imposed on the interwaves is the sum of that due to the waves and the rims (Figure 7.2) . Thus in the case of a 10 μm layer it is $(0.22 + 0.19) = 0.41\text{N}$. This force, divided by the area of the interwaves ($6.0 \cdot 10^{-4}\text{m}^2$) is the stress experienced by the mucus under the interwaves. Consequently this value is 680 N/m² for a 10 μm layer. The calculation of this force forms the first test of this model of locomotion. This is the stress that must be resisted by the solid mucus beneath the interwaves . Is this a reasonable figure when compared to the results of the physical tests?

The ability of the solid mucus beneath an interwave to withstand this stress is dependent on the shear rate that this stress causes. As shown in Chapter 3 this value is difficult to measure from video tape records since a point on the foot moves backwards only very briefly . From video recordings it is only possible to say that the movement occurs in less than 0.08 seconds. The distance a point on the foot moves backwards can be more accurately measured,

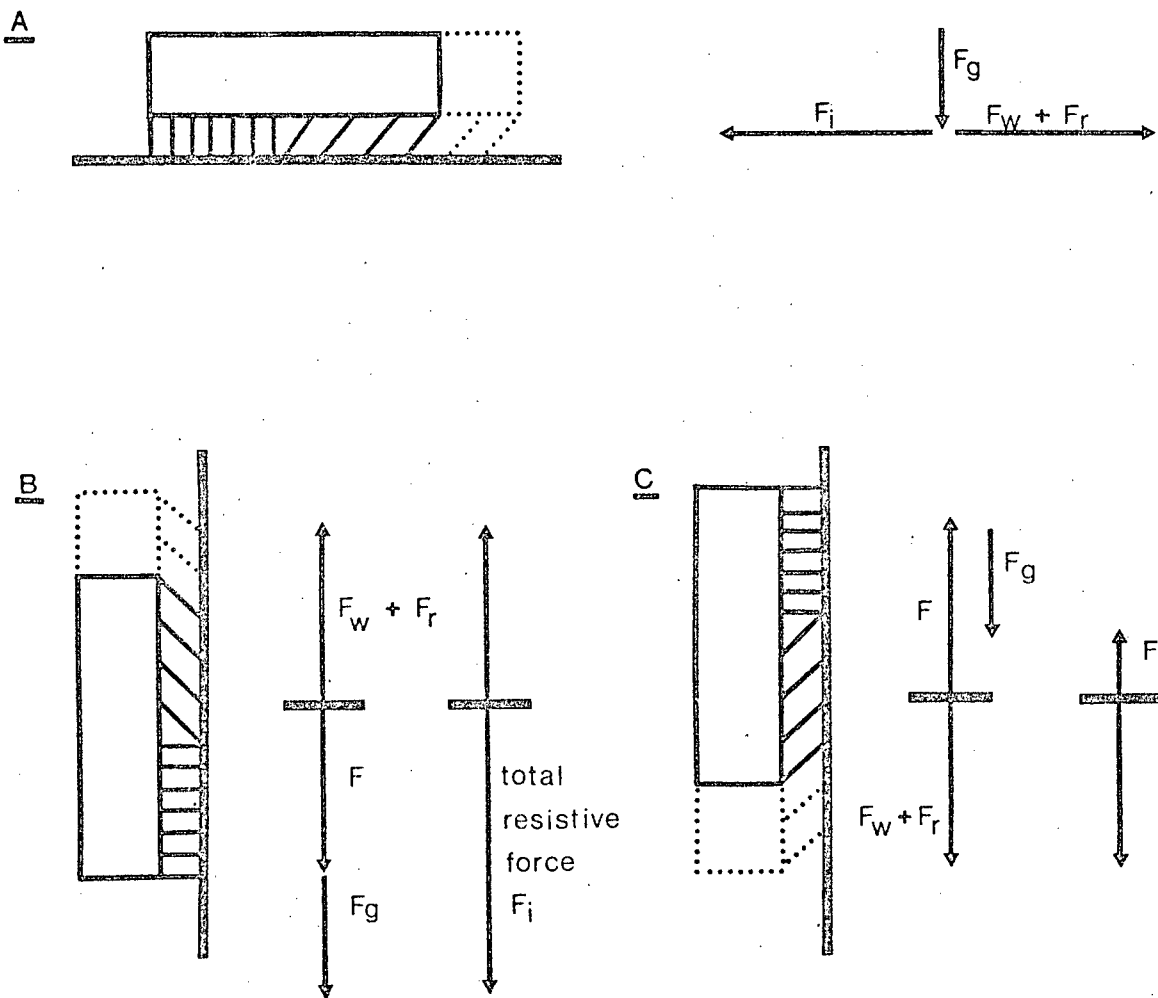
however, and is equal to about 30 μm or less. 30 μm in this case corresponds to a shear ratio for the mucus of 3.0. It is necessary that this shear have been applied at a rate of at least 60/sec in order for the mucus to not yield and become a fluid. At a shear rate of 60/sec the backwards movement of the foot would have lasted only 50 milliseconds, a period too short to have been measured by this technique. It is thus consistent with these observations that the mucus under the interwaves is sheared at a rate sufficiently high to withstand the calculated stress of 680 N/m^2 . After this initial application of force the mucus under the interwaves should creep. This creep however can only occur for the period of time for which a point under the foot is stressed; about one second. It is not strictly valid to equate stress relaxation data to creep data. However, stress relaxation data may provide an educated guess as to how far mucus would creep in one second. It takes solid mucus about 100 seconds to relax to half of its initial stress value. Consequently it may be estimated that mucus would require about the same period to creep to twice its length, or a creep of 0.1 $\mu\text{m/sec}$ for a 10 μm thick mucus layer. This amount of creep is far too small to be detected by the video tests conducted here.

These predictions for the stresses operating under a moving slug apply to a slug moving on a horizontal surface. If the slug is crawling vertically up or down, its weight will apply a force which must be resisted by the solid mucus beneath the interwaves (Figure 7.5) . If in this example a

FIGURE 7.5. The interaction of gravity with the forces of locomotion.

- A) Crawling horizontally; gravity neither aids nor hinders movement.
- B) Crawling vertically upwards; the weight of the slug adds to the force acting on the interwaves.
- C) Crawling vertically downwards; the weight of the slug subtracts from the force acting on the interwaves.

Figure 7.5



slug is crawling vertically up, the force due to its weight (0.15 N) will act over the area of the interwaves ($6.0 \times 10^{-4} \text{ m}^2$) to place an additional stress of 250 N/m^2 on the interwaves. For a slug walking vertically down, this stress due to its weight will be in a direction opposite to the stress caused by the forward movement of foot segments and will subtract from the stress imposed on the interwaves (see Figure 7.5). All the values calculated for the example are summarized in Table 7.1.

The example above refers to a slug moving at a constant 0.85 mm/second . This is a fairly slow speed, even for a slug. Values for the various stresses can be calculated by the method used above for a slug moving at a more rapid speed, for example 2.00 mm/second which is near the upper end of the range of speeds observed in A. columbianus. These values are shown in Table 7.1. As might be expected the stresses present under a rapidly moving slug are predicted to be larger than those under a slowly moving slug, and some values are quite high. If these stresses are to be resisted by the solid mucus under the interwaves, the shear rates caused by these stresses must be very high ($120/\text{sec}$). As explained earlier the accurate measurement of these shear rates was not possible. Is it possible to say whether these shear stresses are compatible with this model?

In answering this question a number of factors must be taken into account. The calculations presented here are based on a number of estimates. A small change in the estimated relative proportions of the different areas of the

foot will have a large effect on the calculated stress values. Use of a different curve for the estimation of viscosity will affect the calculation, as will a difference in the in vivo properties as compared to the in vitro measurements. A contribution by cilia to the force of propulsion would change the calculated values somewhat. With all these possible sources of error it is somewhat remarkable that the calculated interwave stress values correlate as closely as they do to the range of shear rates that is possible and even likely under the foot.

If these interwave stress values are indeed accurate, they give a clue as to why slugs do not travel any faster than they do. The faster a slug walks, the larger the stress on its interwaves. At some speed this stress will become great enough to cause the mucus beneath the interwaves to become a fluid and the slug will no longer be able to crawl. If the interwave stress values calculated here for an animal moving at 2.0 mm/sec seem large, this may simply be a reflection of the fact that at 2.0 mm/sec a slug is walking nearly as fast as its pedal mucus will allow.

A second form of test may be applied to this locomotion model. As explained above this model makes specific quantitative predictions about the stresses present under a moving slug and these forces can be measured using an appropriate apparatus.

Force Plate

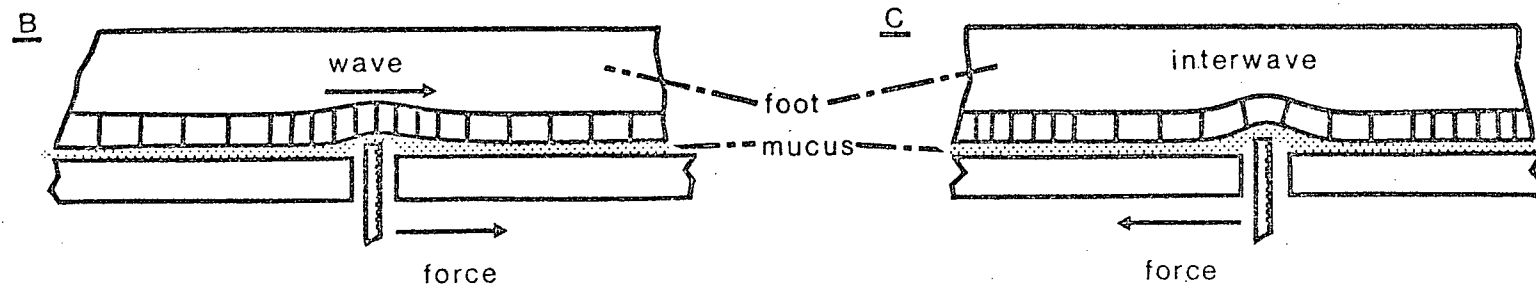
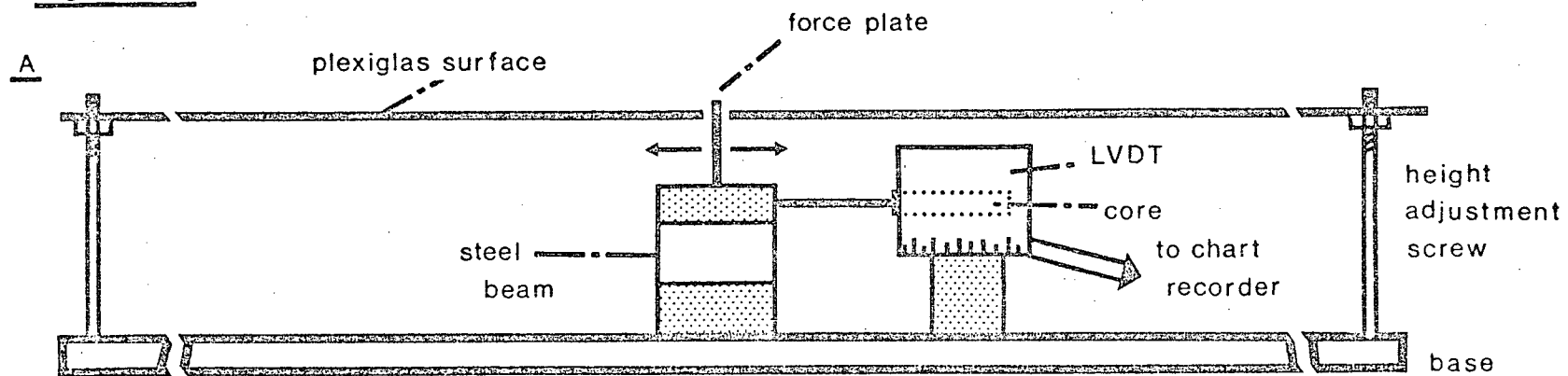
The apparatus used to measure the locomotory forces of

slugs is shown in Figure 7.6. A small (0.5 x 1.0 mm) force-plate protrudes through a 1.5 mm hole in a large plexiglass surface. This force-plate is supported by a double steel beam. A force in the plane of the plexiglass surface will cause the force-plate to move, the larger the force, the larger the deflection. Due to the shape of the supporting beam the force-plate is sensitive to force in only one direction. During a test, the force-plate is oriented so that the shorter dimension (0.5 mm) lies in line with the direction in which the beam is sensitive to force. The amount of deflection of the beam is measured by a linearly variable differential transformer. The output from this transformer is amplified and recorded on a chart recorder. The transducer is calibrated by turning the apparatus on its side (so that the measuring beam is horizontal) and hanging accurately known weights from the plate. Forces as small as about 5 dynes can be accurately measured and within the range of forces encountered during testing the transducer output is linear with force. The unloaded resonant frequency of the apparatus is 100 hz; sufficiently high to allow accurate measurement of the 1 hz oscillatory forces associated with slug locomotion.

A test is performed by orienting the apparatus so that the plexiglass surface is either horizontal or vertical, and then allowing a slug to crawl across the surface (and the force-plate) along the direction in which the force-plate is sensitive to force. The dimensions of the force-plate in this direction (0.5 mm) is less than the shortest length of

FIGURE 7.6. An apparatus for measuring the forces beneath a crawling slug.
A) Schematic representation of the apparatus.
B and C) The dimensions of the force plate are smaller than either a wave or an interwave.

Figure 7.6



a compressed wave. Thus, as a slug passes over the force-plate forces beneath waves and interwaves will alternately be measured (see Figure 7.6). By correctly positioning the slug the force present beneath the rims may also be measured. Since the area of the force-plate is known the measured forces can be expressed as force/area, i.e. stress, for comparison with the prediction of the model. All tests were performed at room temperature (21-23 °C). It has been amply shown by Lissman (1945b) that the passage of a compressional wave corresponds to an anteriorly directed force. This observation was corroborated by observing the ventral surface of the foot as it passed over the force plate, and was not tested further.

This apparatus is less than ideal in two respects:

1. Because the force measuring plate must be able to move in order to measure force it must protrude through a hole in the plexiglass surface. The presence of a hole means that the slug can lift its foot as a wave passes over the plate. Thus as long as the force-plate is flush with the surface only the forces beneath the interwaves (where the foot is not lifted) will be measured. Further, unless the force-plate is precisely flush, these forces will not be measured accurately. This problem is remedied by raising the plate above the plexiglass surface slightly (50 μm to 150 μm). This maintains the contact between the foot and the force-plate at all times. Apparently, while the slug can lift its foot in the general region of the hole, it cannot lift it sufficiently in the localized region of the

force-plate to break contact with the force-plate. Once the force-plate is raised sufficiently to maintain contact with the foot the distance that it is raised (within the limits 50-150 μm) does not appear to effect the stresses measured. The correlation between force-plate height and overall force amplitude has a correlation coefficient of 0.059, which is far from significant. The distance the plate is raised is measured with a micrometer.

2. The second problem encountered with this apparatus is again attributable to the presence of a hole in the plexiglass surface. It is noted that in a large percentage of tests the stress measured by the plate after the slug has passed is different than the zero stress measured before the slug first reaches the plate. The magnitude and even the direction of this zero shift is unpredictable. Apparently at some point as the slug passes over the plate, solid mucus builds up in the gap either in front or behind the plate. While this zero shift does not affect the overall amplitude of stresses measured, it makes it impossible to measure the relative amounts of this overall amplitude that are directed anteriorly and posteriorly.

As a consequence, test results are divided into two categories. The small percentage of tests where the zero stress level is shifted by less than 10% of the overall force amplitude during the passage of a slug are placed in one category and stresses are measured relative to the zero level. Tests where the zero level is shifted by a larger amount are placed in the second category. In this category

the overall amplitude of the stress oscillation is measured (as shown in Figure 7.7) but no attempt is made to subdivide this overall stress into anteriorly and posteriorly directed stress.

Tests

Two sorts of tests were performed. First with the plexiglass surface horizontal the stresses beneath the waves, interwaves and rims were measured. Second with the plexiglass surface vertical the stress beneath waves and interwaves was measured for slugs walking either vertically up or down. The speeds at which the slugs walked during the tests were not measured. This was due to practical problems involving the perverse nature of slugs. The slug is capable of moving in a manner such that the anterior half of the body is moving at a different speed than the posterior half. Thus, as the slug starts and stops as it moves over the force-plate measuring the speed of either the head or tail may not give an accurate measure of the speed at the force-plate. To accurately measure the speed of the slug in the area of the force-plate it would have been necessary to actually film this area. Without a transparent force transducer this proved impossible. It is assumed that the range of walking speeds in these tests is similar to those shown in Figure 3.3. The results from these two types of tests will be discussed in turn.

Horizontal Tests

The overall amplitude of the stress measured under the central portion of the foot for 34 tests on 19 different slugs was 1493 N/m^2 with a range from 780 to 2820 N/m^2 . This compares quite favorably with the predicted values for overall stress amplitude which range from 830 to 2280 N/m^2 . A list of predicted and measured values is shown in Table 7.2. The values of 5 measurements of rim stress average 350 N/m^2 and range from 200 to 510 N/m^2 . Again these values are very similar to the predicted values. Thus, on the basis of overall amplitude the model's predictions are quite accurate. Does the accuracy also hold for the measurement of posteriorly and anteriorly directed stresses? The records obtained from three slugs had sufficiently small zero shifts to allow for the measurement of anteriorly and posteriorly directed stress. The record of one of these tests is reproduced in Figure 7.7. Anteriorly directed stresses averaged 340 N/m^2 ranging from 290 to 390 N/m^2 . Posteriorly directed stresses averaged 660 N/m^2 ranging from 590 to 740 N/m^2 . Again these values are quite close to those predicted by the model (see Table 7.2).

Vertical Tests

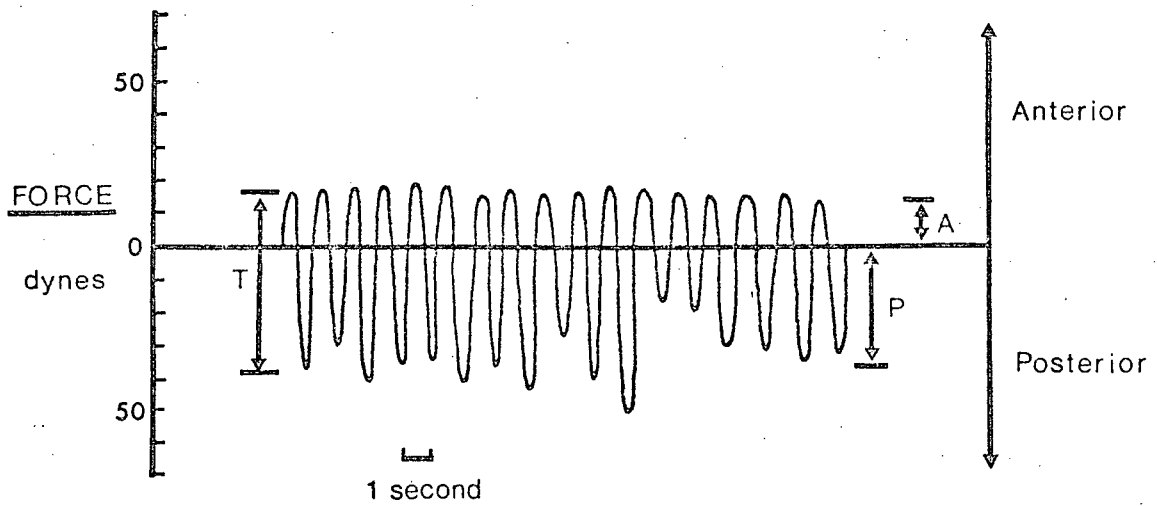
Seventeen tests on 9 slugs were conducted to compare the stress values between crawling horizontally and vertically up. The average value for the overall stress amplitude while crawling vertically up was $1991 \pm 354 \text{ N/m}^2$. This value is significantly greater (p less than 0.01) than the mean of the overall stress amplitude for the

Figure 7.2: The measured and predicted forces of Ariolimax columbianus locomotion.

	STRESS (N/m ²)	
	predicted	measured
rims	297-659	201-512
waves	365-1059	336-526
interwaves	485-1222	591-1171
overall	850-2281	780-2280 $\bar{x}=1493$

FIGURE 7.7. An example of the record of forces measured beneath a crawling slug, with terms defined. Amplitudes are measured for each wave and are averaged for each slug.

FIGURE 7.7



T - total amplitude

A - amplitude of anteriorly directed force

P - " " posteriorly " "

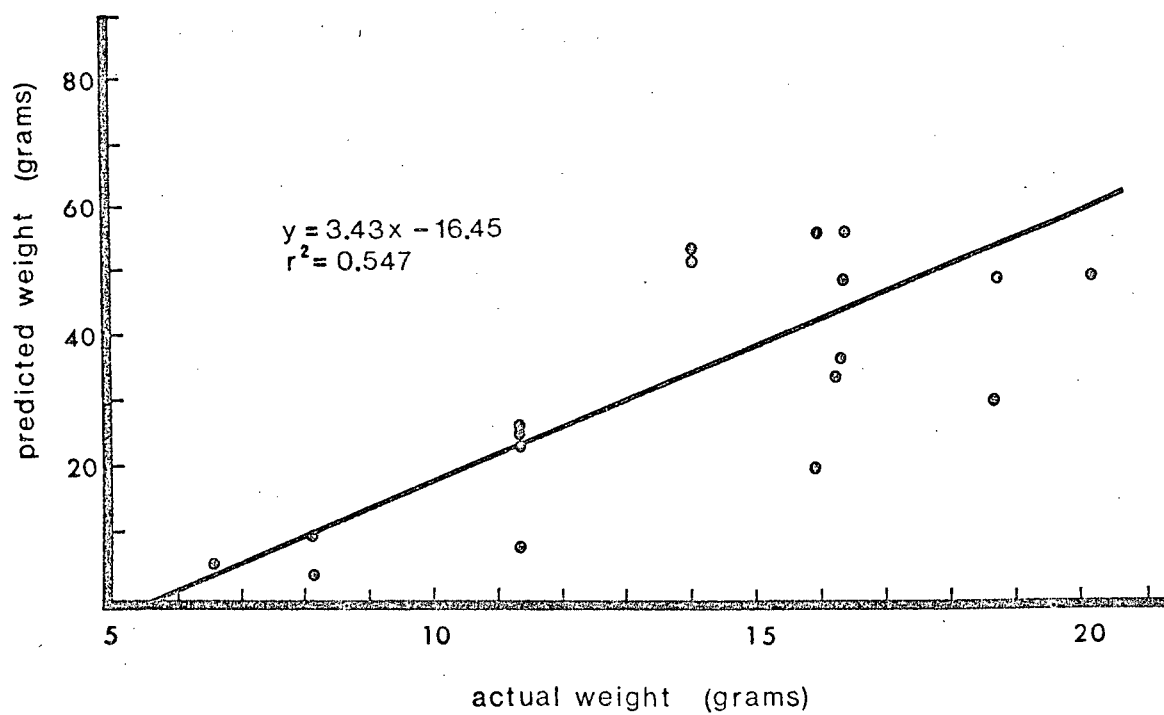
horizontal tests which were conducted concurrently (see below) when compared by a one way analysis of variance.

Each of these 17 vertical tests was followed by turning the plexiglass surface so that it was horizontal and allowing the slug to crawl over the force plate again. The mean of these horizontal tests was 1383 N/m^2 . The area of the foot of each slug was then measured using the video recorder and each slug was weighed. As explained earlier the difference in stress value between walking vertically and horizontally should be equal to the force due to the weight of the slug divided by the area of the interwaves. Having measured the difference in stress, the interwave area, and the slug weight the predicted and actual values can be compared. This is accomplished by plotting the predicted weight versus the actual weight (see Figure 7.8). In theory the result should be a line passing through the origin with a slope of one. It can be seen that, as predicted, the change in stress amplitude (and therefore the predicted weight) increases as the slug's weight increases. However, the change in stress amplitude is significantly greater ($p < 0.05$) than that predicted by theory. In other words, some factor other than just the slug's weight is increasing the force required to move the slug vertically. The basis for this fact is not at present known, though several possibilities exist:

1. A. columbianus is strongly negatively geotactic. Consequently it is possible that the slug would crawl faster as a response to being tilted to a vertical position. While

FIGURE 7.8. A plot of weight as predicted from the model versus actual weight for all vertical crawls. While predicted weight is proportional to actual weight the slope of the relationship is greater than expected.

Figure 7.8



the speed of the slug was not measured during these tests (as explained above), so that this factor cannot be definitely ruled out, no visible increase in speed was noted for slugs crawling vertically.

2. It is possible that the slug alters the area of the rims in response to the angle of the substratum relative to the vertical. Slugs crawling horizontally are often seen to have part of the rims lifted from the substratum. However, when crawling vertically the rims are invariably closely applied to the substratum. This increase in effective rim area would increase the stress amplitude.

3. It is also possible that the mucus layer thickness is altered when crawling vertically. Decreasing the thickness of the mucus layer would increase the force needed to move the slug upwards, but would also make it less likely that once the slug stops crawling it will slide down the surface. Perhaps this advantage of extra adhesive strength offsets the increase necessary in locomotory energy expenditure. This matter certainly warrants further study.

In two tests, in addition to the slug's walking horizontally and vertically up, the slug was induced to walk vertically down. These tests are difficult to conduct as the slugs normal behavior is to crawl up a vertical face. In this case the overall stress amplitude should be less for the vertically down crawling than the horizontal crawl by an amount related to the slug's weight. Again while qualitatively correct, the model overestimates the animal's weight. The stress records from one such series of tests

are shown in Figure 7.9.

Pressures Beneath Pedal Waves

The model presented in Chapter 3 for the kinematics of A. columbianus locomotion predicts that there should be a dorsally directed force on the mucus beneath a pedal wave when the slug is crawling on a non-porous surface. Without a better understanding of the role played by muscles in the propagation of pedal waves, the magnitude of this force cannot be predicted. It should, however, be possible to detect this force, if it is indeed present. To this end the force plate used in the above tests was modified as shown in Figure 7.10. The essential modification consists of replacing the force measuring plate with a hollow tube cut from a 20 guage hypodermic needle. This tube is connected by a rigid plastic tube to a pressure transducer. All tubing is filled with degassed water. Thus, as the slug walks over this new force plate, any force directed dorso-ventrally will be detected by the pressure transducer. Unfortunately the detection of pressure by this transducer is accomplished by measuring the movement of a diaphragm. Consequently there is some volume change in the system accompanying a pressure change. Since the volume changes that would accompany a slug lifting its foot during a pedal wave are likely to be quite small (about .1 to .5 ul for one whole wave, considerably less for the small part of a wave over the tube) the volume change due to pressure detection is likely to substantially affect the pressures being

FIGURE 7.9. A representative record of the forces measured beneath a slug when crawling vertically up, horizontally, and vertically down. The slug weighed 16.24 gm and the vertically up and down records predict a weight of 34.0 and 54.4 gm respectively.

Figure 7.9

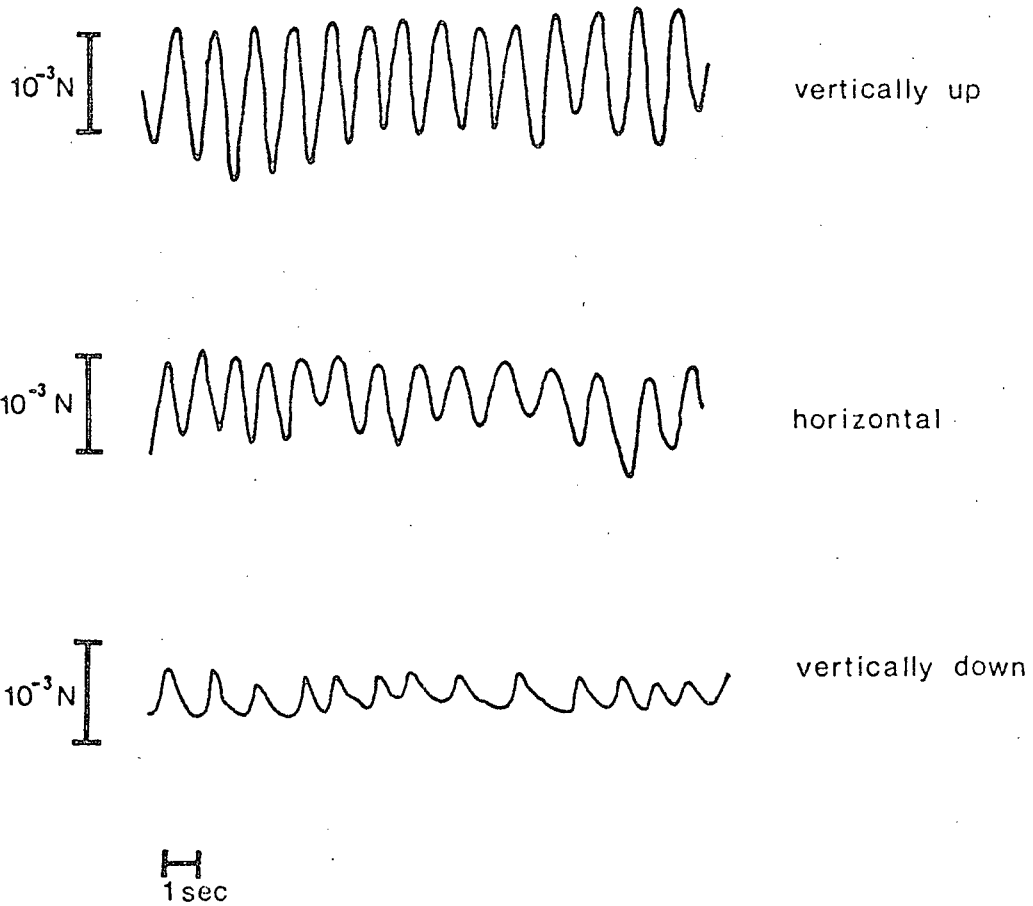
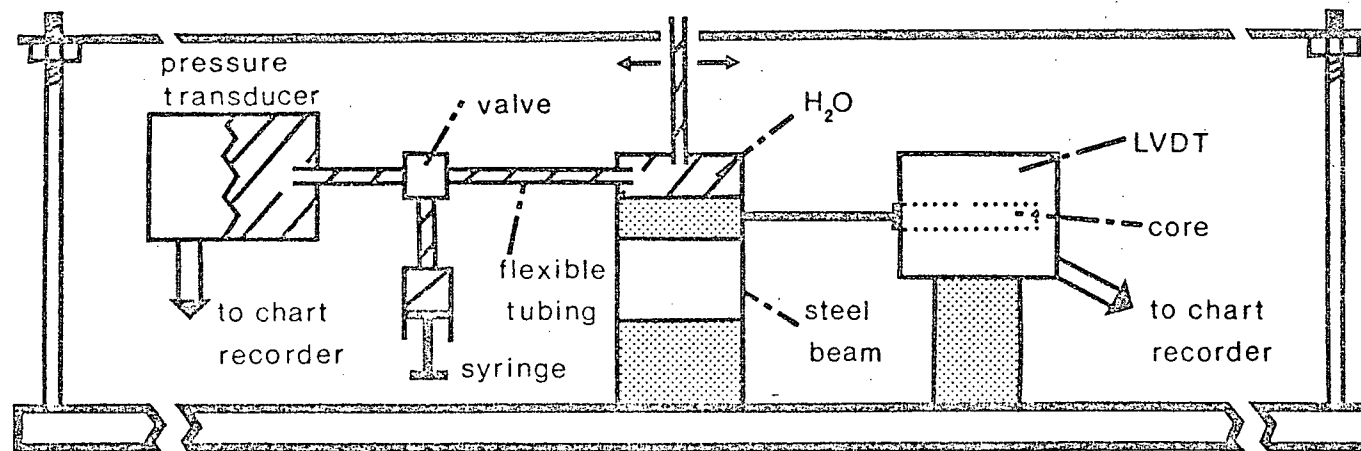


FIGURE 7.10. A schematic drawing of the apparatus for simultaneously measuring antero-posterior forces and dorso-ventral forces beneath a crawling slug. The syringe and valve are used to adjust the level of the degassed water in the system.

Figure 7.10



measured. Thus, the magnitude of pressure measured by the apparatus are probably much too small . The apparatus does however, accurately measure the presence and direction of pressure. In addition to these pressure measurements the apparatus can still measure antero-posteriorly directed forces.

A series of six tests on three slugs confirms that there is a dorsally directed force acting under a pedal wave. The record from one such test is reproduced in Figure 7.11.

Conclusions And Discussion

On the basis of the kinematics of locomotion and the physical properties of pedal mucus a model can be drawn that quantitatively predicts the stresses operating under a moving slug. These stresses can be measured experimentally and the measured stresses compare closely to the predicted stresses.

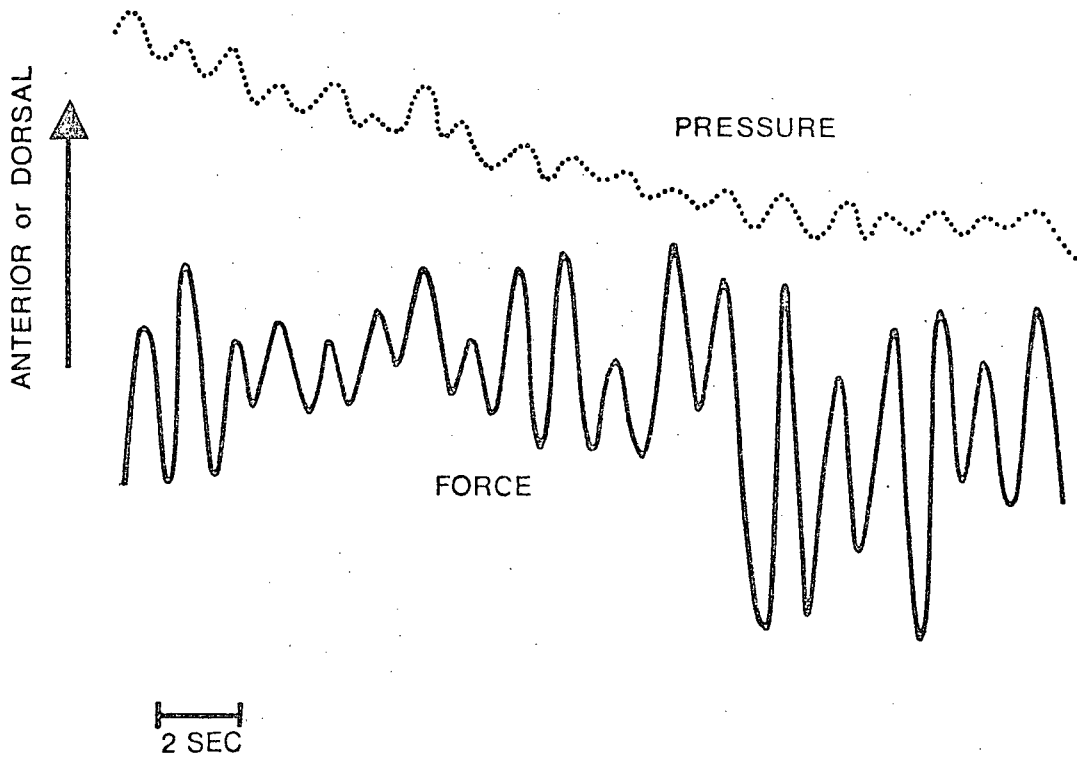
These results lead to two conclusions:

1. The model presented here is in general an accurate description of the process of locomotion in the slug A. columbianus . It appears that the assumptions and simplifications made in constructing the model are justified. More study is needed, however, to account for the discrepancy encountered when slugs crawl vertically.

2. If the properties of pedal mucus measured separate from the foot of the slug provide an accurate description of the properties of this mucus when actually used in

FIGURE 7.11. An example of simultaneous force and pressure measurements. The dorsally directed force peaks at the same time as the anteriorly directed force. The overall slope of the pressure trace is due to temperature drift in the transducer.

Figure 7.11



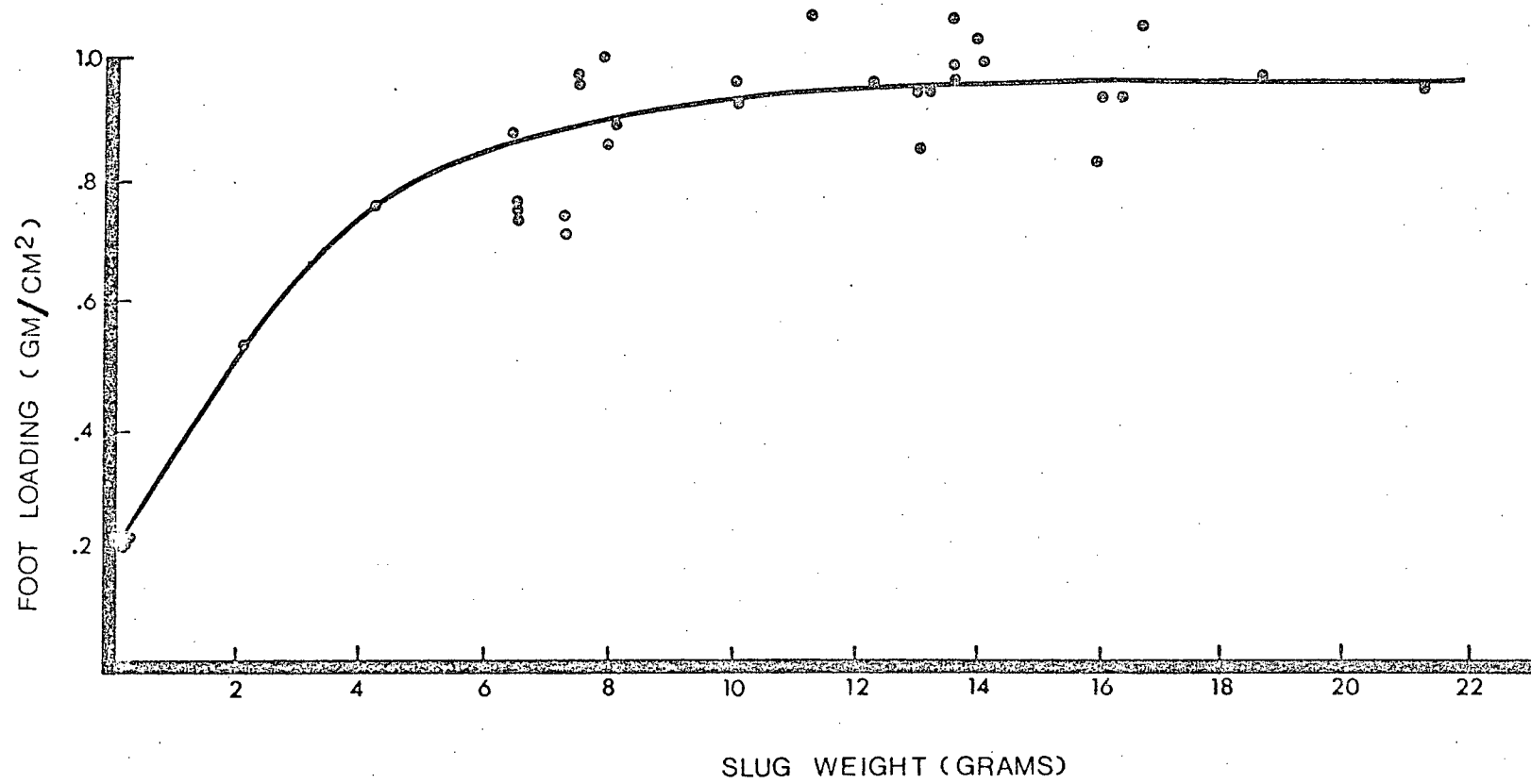
locomotion then it is unnecessary to propose any elaborate mechanism whereby the properties of the mucus are controlled by the pedal epithelium during locomotion. While this model is reasonably accurate as far as it has been tested, I do not wish to imply that it is an all encompassing model for gastropod locomotion. In addition to the discrepancy previously noted for vertical crawling there is at least one aspect of slug locomotion which this model cannot explain, and which deserves further study. As described in Chapter 3, a slug suspended in midair can transport mucus posteriorly along the foot. While waves are present on the foot at these times, no aspect of this model can explain this phenomenon. It seems likely that this mucus transport is a result of ciliary action, but this has not been conclusively shown. Another large area for future study concerns the application of this model or some derivative of this model to the problem of locomotion in animals using retrograde waves. This will be discussed in Chapter 10. The model drawn here was for a "typical" slug weighing 15 grams and with a foot area of 15 cm². How must this model be scaled to apply to slugs of different sizes? Two factors are involved in this problem: 1) The foot area will determine the area of mucus over which the slug must move, and thereby the frictional resistance to movement. Thus, if all slugs have the same shape, foot area may be expected to increase as the square of the length of the slug, L². 2) The mass of the slug will determine the volume of muscle available to do external work, and for a slug crawling

vertically, will also affect the amount of external force required to move. The mass of the slug will be directly related to the volume of the slug, and thus, for a slug of a standard shape, may be expected to increase as the cube of the length of the slug, L^3 . On this basis, the foot loading (mass/foot area or L^3/L^2) would be expected to increase in proportion to the length of the slug. If this is indeed the case it may be necessary to build a scaling factor into the model.

Foot loading values were measured for slugs of a variety of sizes and are presented in Figure 7.12. It can be seen that for slugs above about 5 grams the foot loading remains constant at a value of about 0.95 g/cm². This means that the slugs must change shape as they grow in a manner which maintains a constant ratio between weight and foot area. Thus, the ratio of muscle to frictional resistance of movement may be expected to remain constant, and the model, while calculated for a 15 gram slug, may be directly applied to any A. columbianus of greater than 5 grams, no scaling being required.

FIGURE 7.12. The foot loading (weight/foot area) of Ariolimax columbianus is constant above a weight of about 5 grams.

FIGURE 7.12



CHAPTER 8

Cost Of Locomotion

The preceeding chapter provides a model for the mechanism of locomotion in A. columbianus and an estimate of the forces involved in movement. It would be interesting to extend this knowledge of how the animal crawls, and ask the question: how costly is this form of locomotion? Compared to a fish, a bird, a reptile, or a mammal, how expensive is it for a slug to move itself from place to place? Before examining this question in detail it will be useful to review some basic concepts dealing with the cost of locomotion.

Review Of Terms

As shown in Chapter 7, a moving slug must exert an anteriorly directed force in order to overcome gravity and the viscoelastic properties of the mucus over which it crawls. The result of this force is the movement of the slug through a certain distance. The product of force times distance is work or energy and is expressed in SI units as Joules (J). Energy expenditure per unit time is power, expressed as J/second or Watts (W). Thus the force exerted by an animal as it moves, times the velocity of movement (distance/time) is equal to the power expenditure (force x distance/time = work/time = power). It is useful when comparing costs between animals to normalize the cost to the

weight of the animal. Thus the power required to move at a given velocity will be expressed as W/kg .

The power necessary for locomotion can be divided into two categories. The first of these is the power predicted by the model in Chapter 7. The force predicted by the model is exerted by the animal on its surroundings, the mucus and the substratum. This force, times the slug velocity at which it is exerted, provides a measure of the power expended in overcoming factors external to the slug and is termed the external power (P_e). In contrast there is the internal power, P_i , the power that must be expended inside the slug's body to cause work to be done on the environment. The internal power is the sum of several parts. First, the forces of locomotion are a result of muscular contraction. Energy, in the form of ATP, must be provided to the muscles for contraction to occur. The production of ATP from the slug's food is far from being a 100% efficient process. Consequently the animal must spend more energy in providing ATP to the muscles than will appear as external work. Further all work of contraction of muscles is not directly applied to locomotion. For example, muscles must contract to maintain the hydrostatic skeleton during movement, and muscles of the foot expend energy as they move the fluid of the pedal haemocoel. Again these factors (and others like them) increase the amount of internal energy that must be expended to result in a given amount of external work. Finally, energy must be expended in constructing the glycoprotein of the mucus, an internal expenditure that does

not result in any external force. As a result of all of these factors the total internal power will be greater than the external power. The ratio of the two, P_e/P_i , is one measure of the efficiency of the locomotory process.

As mentioned above the external power required for slug locomotion can be accurately estimated for any speed using the model of Chapter 7. The internal power is less directly calculated. The energy required for the internal expenditures of locomotion is ultimately provided by the oxidation in the slug of various energy storage compounds; glycogen, lipids, and protein. The external characteristics of this oxidation are a production of CO_2 and a consumption of O_2 . Depending on the precise compound being oxidized a given amount of CO_2 will be produced, and O_2 consumed, for each Joule expended by the animal. Thus by measuring total CO_2 production and O_2 consumption and using the proper conversion factors it is possible to estimate the total internal energy expended by the animal at any one time. A large portion of this total energy supply will be used simply in the maintenance of the animal and is not directly related to locomotion. Consequently the internal power of locomotion is estimated by measuring the increase in CO_2 production and O_2 consumption above resting levels that is associated with movement, and from these calculating the increased number of Joules expended by the animal (see Schmidt-Nielsen, 1972).

With these terms and concepts in mind, an experiment was designed to simultaneously measure O_2 consumption,

distance crawled, and rate of crawling in A. columbianus. From these data and the model of Chapter 7 three factors were calculated.

1) The cost of moving a certain distance (expressed as Joules /kg m).

2) the power (both internal and external) expended to move at a given velocity, and

3) the efficiency of locomotion.

Apparatus And Experimental Protocol

A differential electrolytic respirometer was constructed to measure the O₂ consumption and CO₂ production of crawling A. columbianus (see Figures 8.1 and 8.2). This respirometer was designed on the same basic principle as that of Close, Unwin and Brown (1978). The animal is housed in a 1.9 liter airtight box containing a CO₂ absorbant (concentrated KOH). As the animal metabolizes, O₂ is consumed and CO₂ produced. As the CO₂ is absorbed the pressure in the test chamber decreases relative to a reference chamber. The reference chamber is identical to the test chamber except that the slug is replaced by an equal volume of water. The test and reference chambers are separated by a thin rubber diaphragm . Mounted on the diaphragm is a small mirror of silvered mylar. As the pressure in the test chamber decreases the diaphragm is bent and the orientation of the mirror is altered. This change in orientation deflects a light beam. The movement of the beam off of an electronic photodetector causes a relay to

FIGURE 8.1. A schematic diagram of the electrolytic differential respirometer used to measure the metabolic rate of slugs. The test and reference chambers are submerged in a constant temperature bath.

FIGURE 8.1

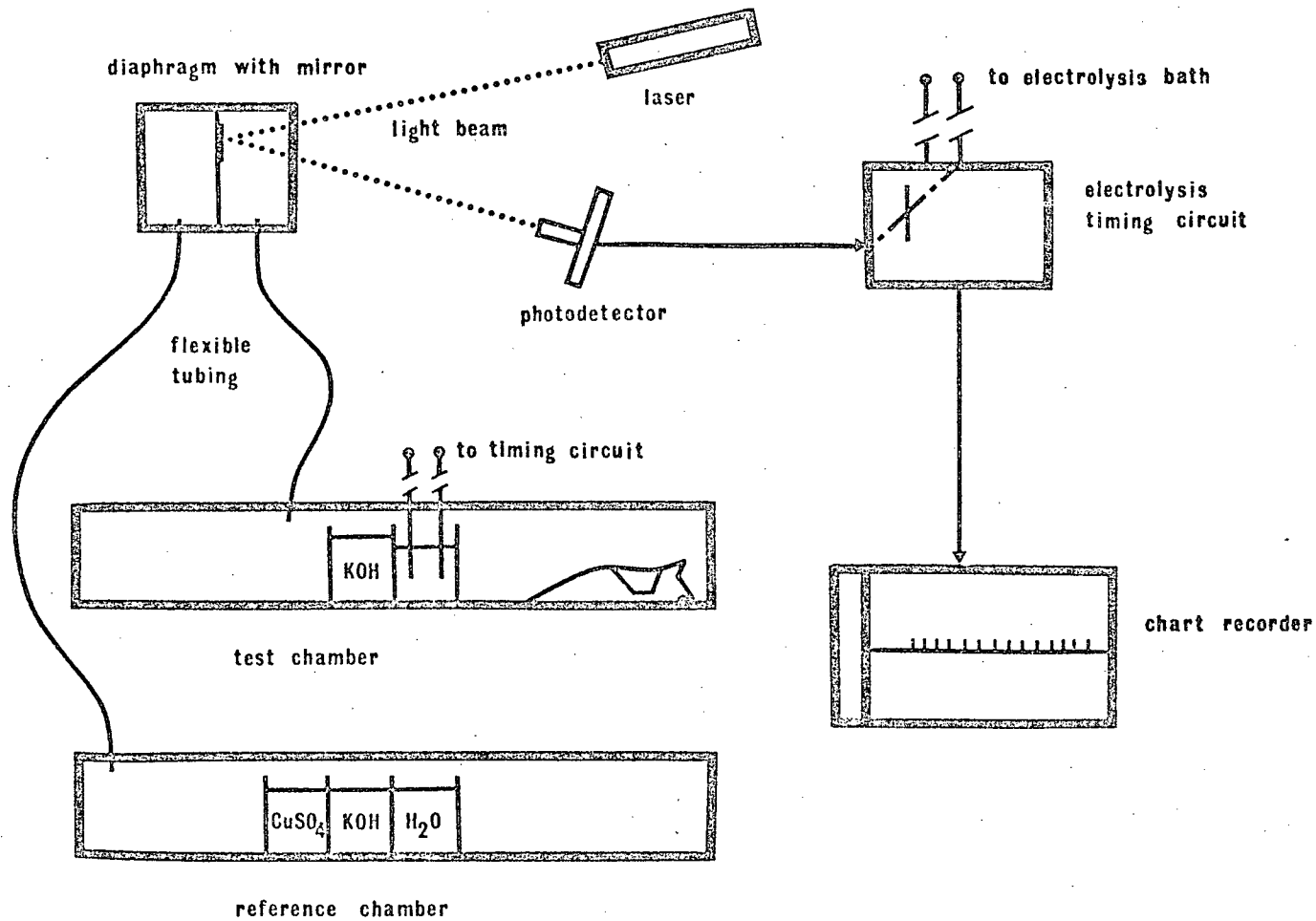
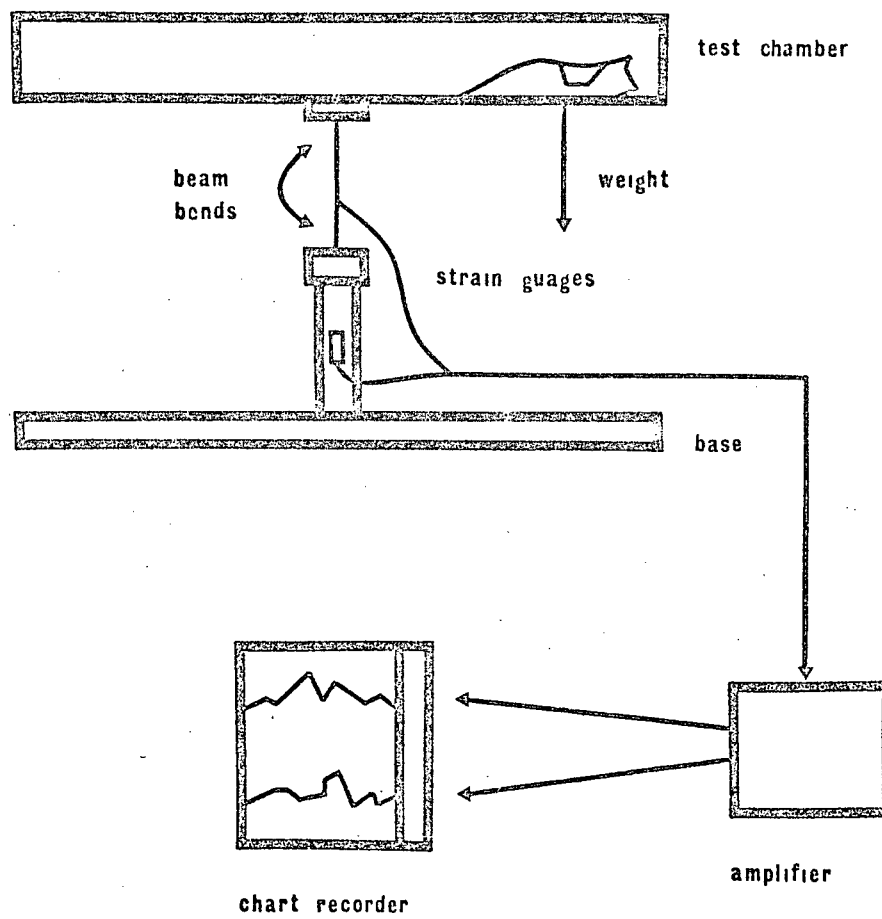


FIGURE 8.2. A schematic diagram of the apparatus used to measure the movement of a slug in the test chamber. The chamber is supported by a compound beam consisting of two orthogonally arranged beams. Each of these single beams is designed to bend in one direction only. The farther the slug is from the center of the chamber along the bending axis of a beam, the more that beam is bent. Thus, strain gauges responding to the degree of bending in each beam provide a measure of the slug's center of mass.

FIGURE 8.2

close and current to be passed through an electrolysis cell in the test chamber. The electrolysis of the CuSO_4 solution in the cell releases O_2 into the test chamber. When this O_2 has replaced the O_2 consumed the diaphragm will have returned to its unbent position, the light beam will again fall on the photodetector and the current to the electrolysis cell will be cut off. The electrolysis circuit contains a timer such that current is supplied to the cell in discrete pulses of a known duration. A chart recorder coupled to the electrolysis circuit records the amperage of each pulse and thereby is a measure of the number of coulombs delivered to the cell. From this, a time record of the amount of O_2 consumed by the experimental animal can be calculated. Both the test and reference chambers are submerged in a constant temperature bath at $19.5 \pm .01$ C. The sensitivity of the apparatus is such that a consumption of 0.01 ml of O_2 can be detected.

The test chamber is supported by a central beam and as the slug crawls about in the chamber its weight causes the beam to bend. Strain gauges glued to the beam measure this bending along orthogonal axes. The amplified output from the strain gauges is recorded on a two channel chart recorder, and provides a continuous record of the location of the slug's center of mass. From this record the total distance moved by the center of mass and the velocity of movement can be calculated. Movement of the slug up or down the side walls of the chamber are not recorded, but due to the flat shape of the chamber, vertical movements are likely

to be small compared to horizontal movements.

A. columbianus in this apparatus showed a distinct diurnal rhythm in movement, remaining stationary during the day , and moving for 3 to 4 hours in the middle of the night. By continuously recording respiration rate and movement over a 24 hour period the resting and active metabolic rates could thus be determined. Crawling velocities under these conditions were quite slow, averaging about 2.2×10^{-4} m/sec, roughly one tenth the slug's maximum crawling velocity.

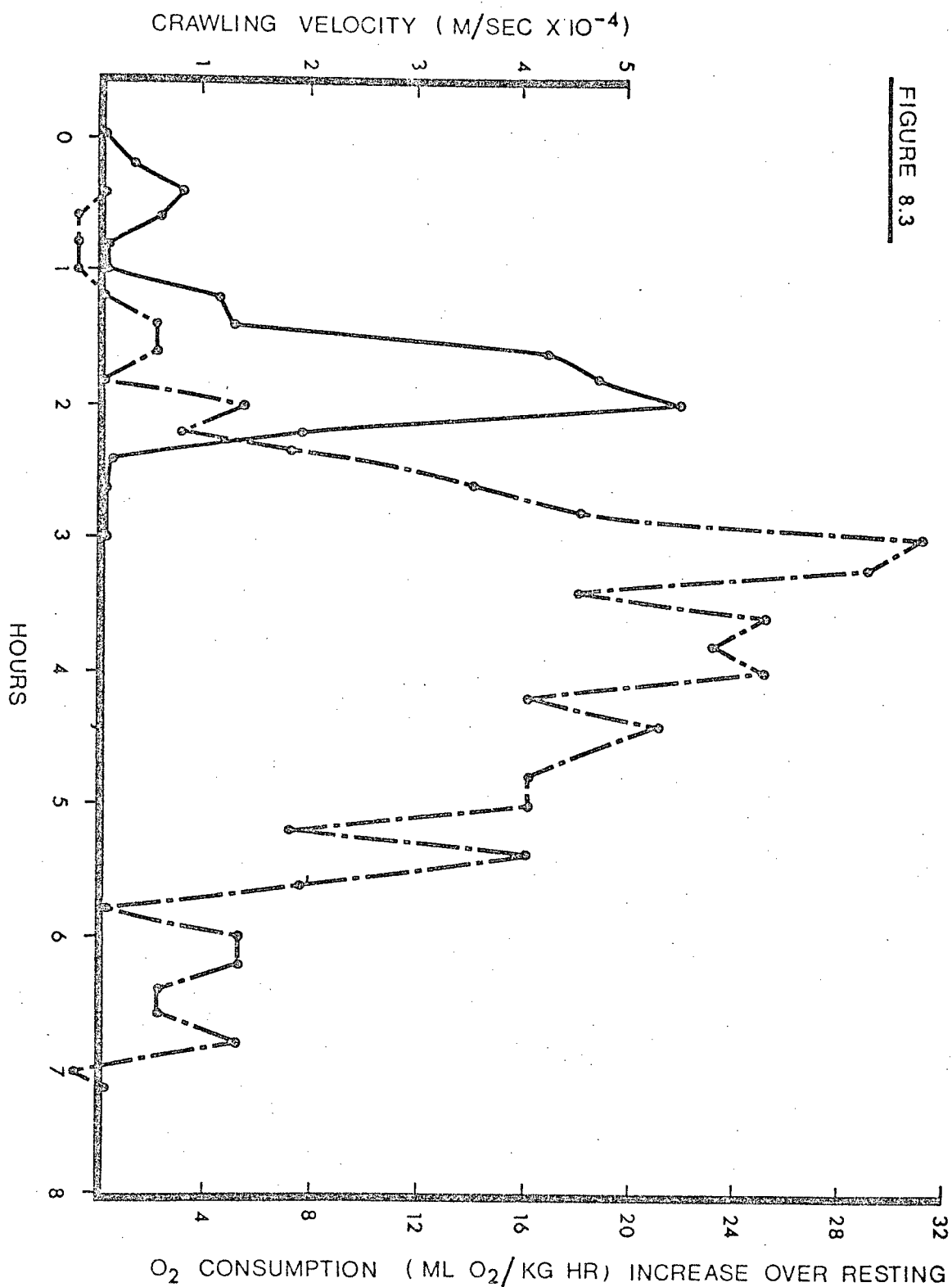
The respiratory quotient (RQ) for A. columbianus was measured by comparing the apparent rate of O₂ consumption for a slug in a test chamber without CO₂ absorbant compared to the same slug in a test chamber with CO₂ absorbant. The RQ thus measured was 0.92, an indication that the slug is using primarily carbohydrates as its fuel. In accordance with this RQ measurement a value of 20.9 J/ml O₂ was used in calculating internal power (Prosser and Brown, 1961).

Results

A typical example of a respiration and movement record is shown in Figure 8.3 . Three facts are worth noting. First, the slug does not move at a constant velocity. Second, the peak in O₂ consumption lags the peak in crawling rate by approximately one hour. This delay in observed O₂ consumption is probably due to three factors: 1) Given the inefficiency of the circulatory and respiratory systems in slug (as compared for example to a mammal) it seems likely

FIGURE 8.3. A representative record of crawling velocity and oxygen consumption for Ariolimax columbianus. Note that the slug does not crawl at a uniform speed, and that the oxygen consumption lags the peak in rate of movement and continues for several hours after movement ceases.

FIGURE 8.3

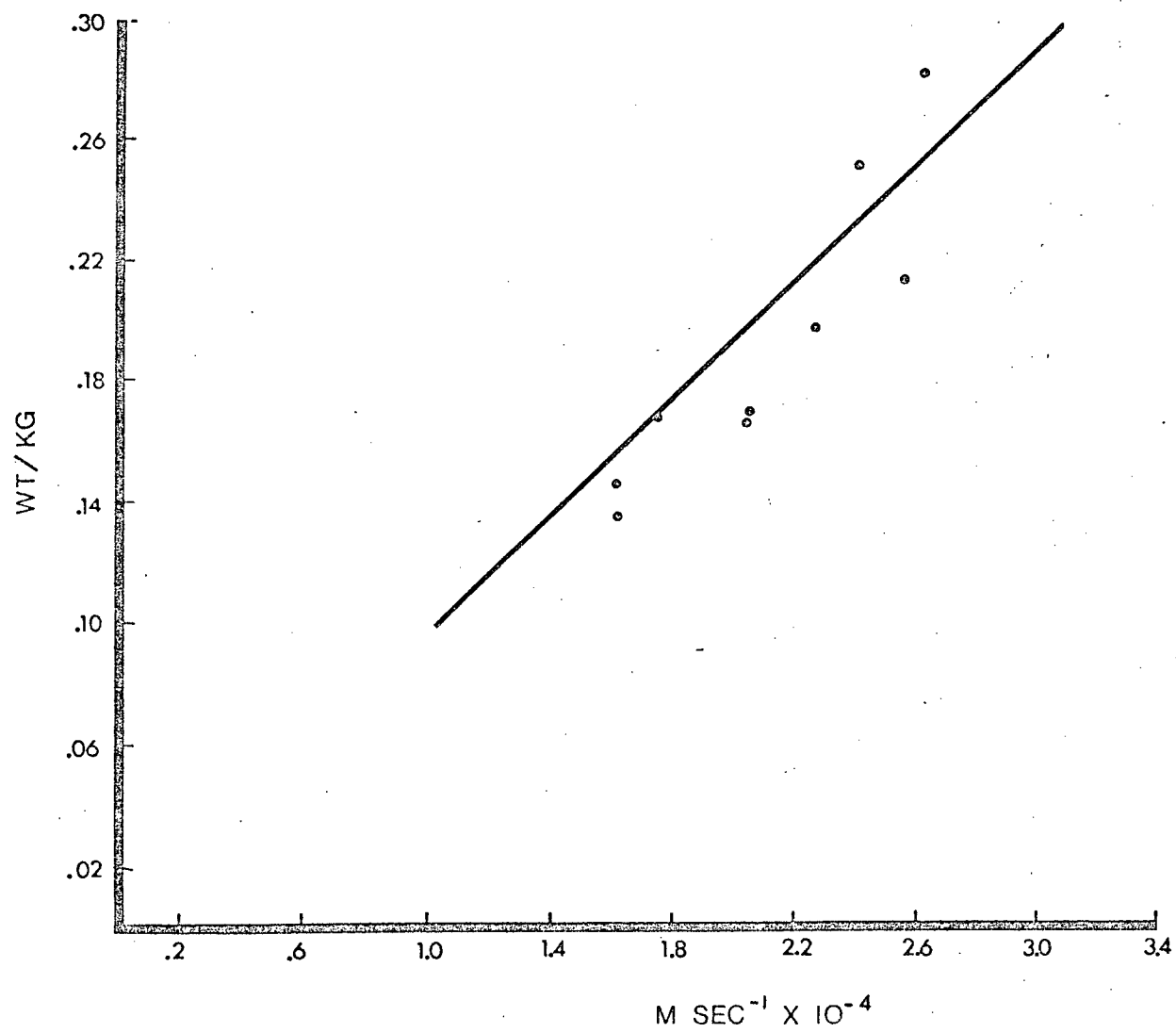


that an energy expenditure in the locomotory musculature may result in an oxygen debt which will take a considerable time to be realized as CO₂ excreted into the atmosphere. 2) The switching on of mucus production, and thereby the metabolic cost of production, may lag the onset of crawling. 3) The absorption of CO₂ by the KOH bath will not be instantaneous. The third fact to note from Figure 8.3 is that the increased respiration rate due to movement continues for several hours after movement has ceased. This may well be due to any or all of the factors cited above for the initiation of the time lag. As a result of these facts it is difficult to precisely correlate each peak in increased respiration with a specific velocity. Consequently these data were analyzed as follows: for each bout of movement the total distance moved, the total time of movement, and the average velocity were determined. The area under the peak in increased respiration rate (as shown in Figure 8.3) was taken as a measure of the total O₂ consumed in moving through that distance. This O₂ consumption (converted to energy and expressed as J/kg) is a measure of the total internal energy expenditure, and when divided by the time of movement yields the internal power in W/kg to move at that average velocity. Similarly the internal energy, J/kg, divided by the total distance moved, in meters, gives the cost of movement in J/kg m.

The results from 9 measurements on 7 different slugs are shown in Figure 8.4. These slugs ranged in weight from 8.1 to 23.3 grams. It can be seen that the power necessary

FIGURE 8.4. The internal power of locomotion as a function of crawling speed for Arioloimax columbianus. The slope of the line is the average cost of locomotion (952.5 J/kg m).

FIGURE 8.4



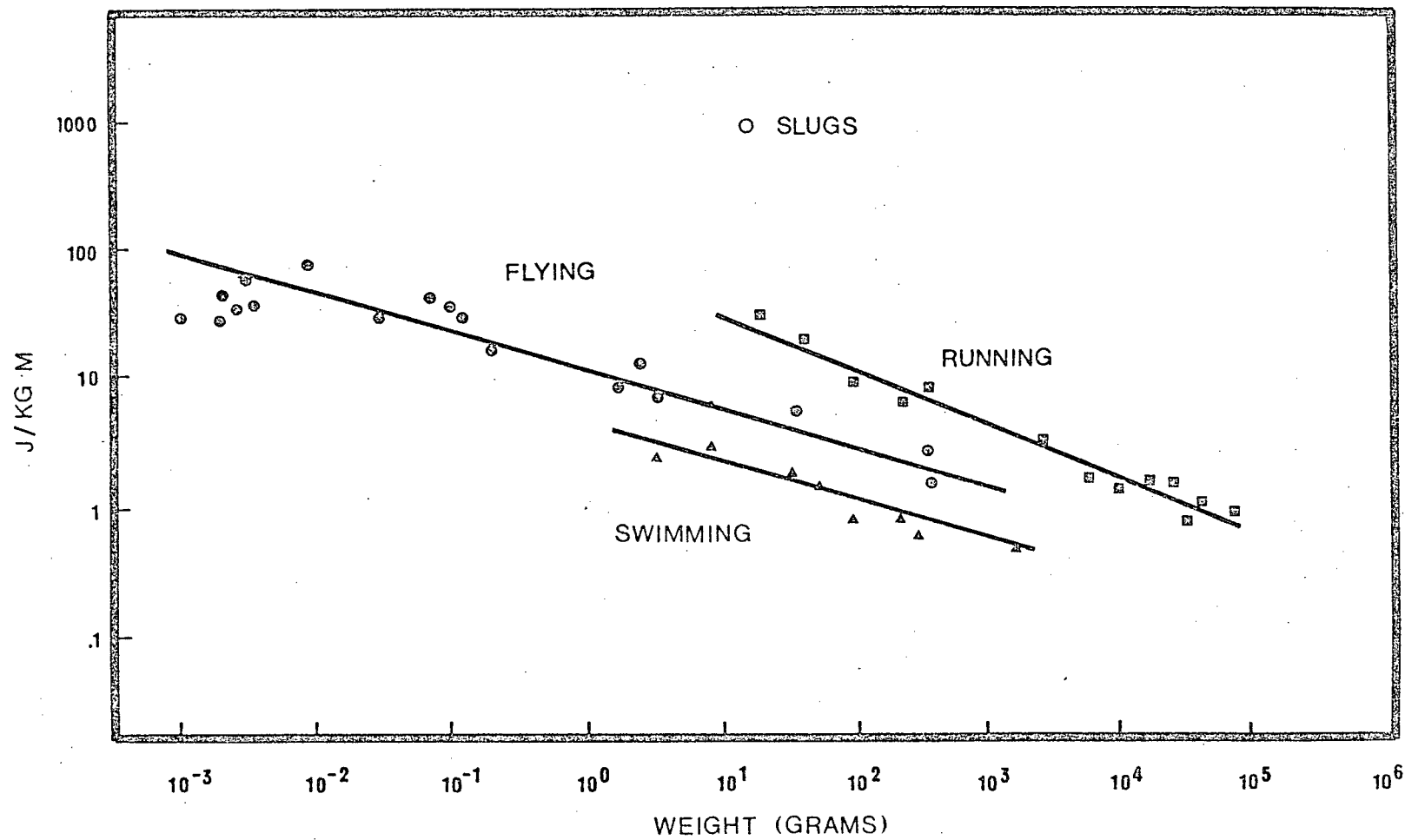
for movements increases with increasing speed. Since W/kg divided by m/sec equals $J/kg\ m$ the slope of the line through these points equals the average cost of locomotion which is $952.5 \pm 130.6\ J/kg\ m$.

The method of calculating cost of locomotion and power used here differs from that used by other authors (see Goldspink and Alexander, 1977). The typical procedure is to induce an animal to move at a constant rate and then measure the steady state O_2 consumption. Since the slugs in this experiment could not be persuaded to walk at a constant rate, the measurement of steady state O_2 consumption was not possible. The total O_2 consumption values used here, in that they include O_2 consumed after locomotion has ceased, are likely to be somewhat higher than if steady state values could be measured. They are however an accurate measure of the cost of locomotion.

Keeping this fact in mind we can now compare the cost of locomotion in slugs to that of other animals. This comparison is facilitated by the generalizations presented by Tucker (1971), and Schmidt-Nielsen (1972) (for an excellent review of this literature see Goldspink and Alexander, 1977). These authors plotted the log of the cost of locomotion against the log of body weight and found a number of surprisingly simple relationships (see Figure 8.5). The most striking of these is that all those animals which move in a similar manner fall on a single line. Thus the data points for all swimming animals may be linked by a straight line, and similarly for animals that fly, and

FIGURE 8.5. The cost of locomotion (redrawn from Goldspink, 1977, after Schmidt-Nielsen, 1972). For any given weight it is more costly to fly than to swim; and run than fly. The cost of locomotion measured for a slug is considerably greater than any value previously measured.

FIGURE 8.5



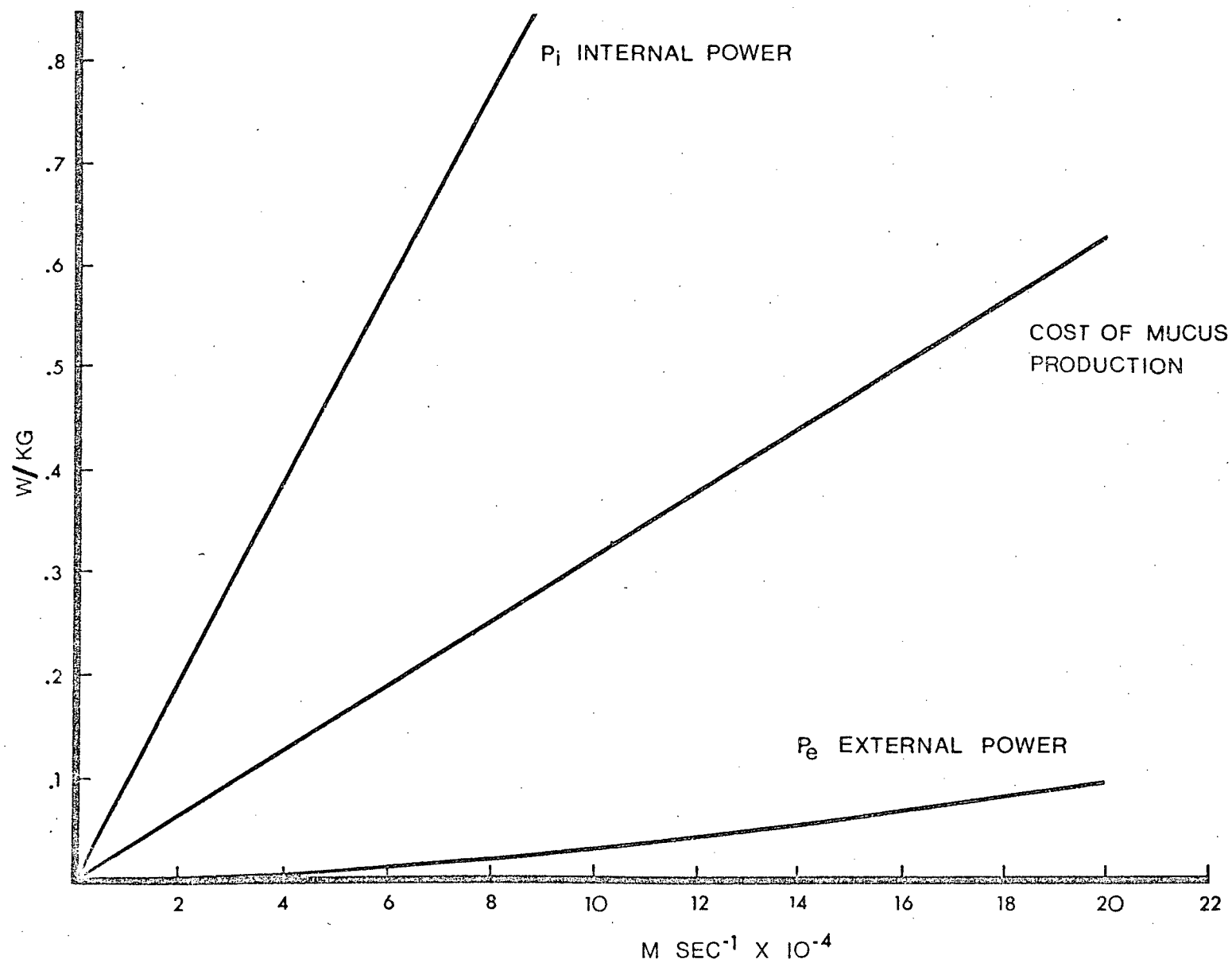
animals that run. The second pertinent fact is that there are sizable differences in cost between the types of locomotion. For an animal of a given weight, the cost of moving 1 kg of that animal 1 meter is least if it swims, more if it flies, and greatest if it walks. This implies that regardless of the fine details about how an animal moves, its basic and most general pattern of movement ties it, in a metabolic sense, to other animals that show basically similar patterns. In this context then we can examine the open circle on Figure 8.5 which represents the cost of locomotion in the slug. It is immediately apparent that the slug's form of adhesive locomotion is a very costly way to move. Even allowing for the fact that the estimate of cost for a slug may be slightly high due to the difference between steady state and total O₂ consumption, it will cost a slug nearly ten times as much to move a given distance as a running animal of equal size.

Why is this so? Using several means of approximation it is possible to examine the various components of the energy expenditures of the slug and thereby arrive at a better idea of what it is about adhesive locomotion that makes it so costly.

Using the locomotion model of Chapter 7, the external power of movement on level ground can be calculated for several velocities and for a mucus layer 10 μ m thick. These are plotted in Figure 8.6. The slope of this curve ($W/kg/m/sec = J/kg\ m$) (the cost of movement) increases with increasing velocity. This is as one would expect from

FIGURE 8.6. The components of power in the locomotion of Ariolimax columbianus. The estimated external power, P_e , forms only 1.7% to 5.2% of the internal power, P_i , measured from respiration studies. The estimated power of mucus production is several times larger than the external power.

FIGURE 8.6



trying to move over a viscous liquid; the faster the rate of movement, the greater the resistance and the greater the cost. These calculated values for external power can be compared to the measured internal power to arrive at a value for efficiency. At 2×10^{-4} m/sec (the rate at which slugs walked in the respirometer) the power needed to overcome the mucus resistance is 16 J/kg m, yielding an energy efficiency value of 1.7%. If the cost of locomotion measured here for slow speeds is assumed to apply to higher velocities, the efficiency at the large slugs maximum velocity of about 2×10^{-3} m/sec will be 5.2%. While these sound quite low they are in reality reasonable values for movement on level ground. For comparison, a human being running on level ground will show an efficiency of between 2.0% and 3.2% (Tucker, 1973). Perhaps a better comparison is that of muscle efficiency alone. While the efficiency of invertebrate muscles has not been measured, it is likely that it does not differ substantially from maximum efficiency of various vertebrate muscles which has been shown to be 20 to 25% (Goldspink and Alexander, 1977). To reach this efficiency the muscle must be contracting at an optimum rate against an optimum load, and under other circumstances the efficiency will be considerably lower. Still, even if 20 to 25% efficiency may be the maximum expected there is still a large gap when compared to 1.7 to 5.2%. How much of the inefficiency of a walking slug can be attributed to the production of mucus? At present this question cannot be answered with any certainty, but

reasonable assumptions can be made to arrive at an estimate. A 15 gm slug with a one centimeter wide foot, and a 10 μ m thick mucus layer will expend 0.1 ml of mucus for each meter it crawls. If this mucus is assumed to be 3% glycoprotein, the amount of protein and carbohydrate expended per meter can be calculated (1.0×10^{-5} gm protein, 6.3×10^{-6} gm carbohydrate). The problem is then to estimate the metabolic cost of producing this amount of protein and carbohydrate. It seems reasonable to assume that the maximum energy expended in producing the polysaccharides will be similar to the cost of producing glycogen from pyruvate. This cost is estimated from Atkinson (1977) at 3099.3 J/gm. Similarly the cost of producing the protein can be estimated at a value of 2704.1 J/gm (from Atkinson, 1977). From these estimations the cost of producing the glycoprotein to replace that lost as the slug crawls can be estimated as 313 J/kg m for a 15 gram slug, and this is independent of velocity. This value represents about 32.5% of the overall energy expenditure of crawling. Thus, when compared to the external work of locomotion, the cost of mucus production may well be the dominant factor.

If it is assumed that the intrinsic maximum efficiency of the muscle in a slug is similar to that in vertebrates (20%) the minimum overall internal cost of muscular contraction directly leading to external work can be calculated simply by multiplying the cost of external work by five. Thus the minimum internal cost of effective muscular contraction during locomotion varies from 8.5% of

the overall cost at a crawling rate of 2×10^{-4} m/sec to 26% at the maximum crawling rate of 2×10^{-3} m/sec. The cost, added to that of mucus production accounts for an estimated 41.0% to 58.5% of the overall internal cost of locomotion.

Where does the rest of the internal energy go? As mentioned above there are a number of internal mechanisms which require energy but do not directly result in external forces. As shown in Chapter 2 the muscles of the foot do not contract in the plane of the foot. Thus for each unit of force which is directed anteriorly as a muscle contracts a roughly equal force will be directed dorsally, doing no effective external work. Further, in addition to the force necessary to overcome the resistance of the mucus, the pedal muscles must also exert a force to overcome the internal viscosities of the foot, for example, the viscous resistance of pumping haemocoelic fluid as described in Chapter 3. Muscular energy will also be expended in maintaining the slug's posture during locomotion. Finally there is the strong probability that the slug's muscles will not all be working at their maximum efficiency. The speed of contraction and load against which each pedal muscle is contracting will vary as waves pass along the foot, and consequently each muscle cannot be continuously contracting optimally. All of these factors and others like them, will account for the remaining expenditures of internal energy.

In conclusion we return to the original question of this chapter: how costly is adhesive locomotion? The answer is: very costly. The estimated cost to a slug of producing

mucus alone is greater than the total cost of movement in a mammal or reptile of similar weight. It seems likely that this high cost of movement will effect the "life style" of these animals, for example, by limiting the distance over which it is profitable to crawl in search of food. However, this high cost of transport must be weighed, in an evolutionary sense, against the advantages to a slug of being able to adhere to the surface over which it walks. Finally while the cost of movement for A. columbianus is quite high, it is not because the mechanism of locomotion is inefficient. Certainly a crawling slug is no less efficient than a running person. Instead, the high cost of transport is dictated by the requirements of the locomotory mechanism: the need to continually produce mucus, and the necessity of working against the viscoelastic resistance of that mucus.

Further studies of the energetics of locomotion in this animal would be useful and informative. For example, in running animals the efficiency of movement increases as the animal switches from moving on level ground to running up a grade. This is due, in large part, to the fact that the increased cost of moving uphill is less than the increase in external work performed as the animal lifts itself against gravity (see Tucker, 1973). It will be interesting to see if the same phenomenon occurs in slugs, animals which spend a large portion of their locomotion time crawling. If this is indeed the case, the efficiency figures calculated here may be considerably lower than those relevant to the animal in its natural habitat.

CHAPTER NINE

Adhesion

The locomotion of gastropods is characterized by the ability to move while adhering to the substratum. The preceding chapters in this study have dealt with the mechanisms of movement of A. columbianus but very little has been said concerning the mechanism of adhesion operating in this animal. What are the adhesive capabilities of A. columbianus , and how can they be accounted for?

The body form of A. columbianus renders an accurate measurement of adhesive strength extremely difficult. In order to determine adhesive capabilities one must be able to exert a force on the adhesive apparatus. However, with a slug, there is no solid structure which can be grasped to exert a force on the animal. Glues will not stick to the mucus coated dorsal epithelium, and the body wall is so weak that sutures cannot be used to attach weights to the slug. Given these limitations one is for the most part confined to general qualitative observations of the slugs' ability to adhere.

If a slug is placed on a sheet of glass or a similar smooth surface, allowed to adhere, and an attempt is then made to detach the slug from the substratum three facts soon become apparent:

1. It is very difficult to pull the animal in a direction perpendicular to the surface.

2. On the other hand it is relatively easy to slide

the slug along the surface.

3. If, by using a fingernail or sliding the slug to the edge of the glass, one small portion of the foot can be lifted from the surface, the whole animal can then be easily peeled off, much as one would peel off a piece of tape.

These observations may be adequately explained by existing theories of adhesion.

THEORY

Adhesion is the ability of two objects to remain attached to each other. Take, for example the situation depicted in Figure 9.1. Two discs, each 1 cm in radius, are arranged co-axially with a gap separating them. This gap (of thickness y) is to be filled with an adhesive. The strength of the adhesive may then be measured by determining the force required to separate the two discs. The separation of the discs may occur in either of two ways:

1. The discs may slide past each other (as in Figure 9.1b). In this case the thickness of the adhesive, y , remains constant but the x coordinate of the discs will vary with time.

2. The discs may be pulled apart axially as in Figure 9.1c. In this case the x coordinate of the discs remains constant but the thickness changes with time.

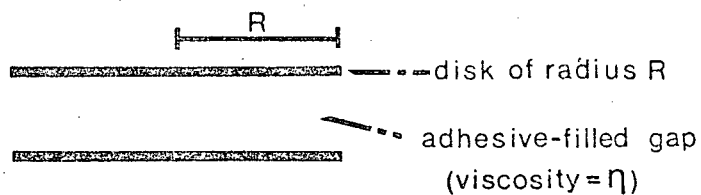
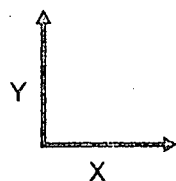
As a first example, imagine that the discs are immersed in fluid with a known viscosity, n . This fluid will fill the gap and act as an adhesive. In the case of sliding one disc past the other the force of adhesion can be calculated

FIGURE 9.1. The adhesive properties of a viscous liquid.

- A) The dimensions of two disks immersed in a viscous liquid.
- B) The force required to slide the disks relative to each other is proportional to the area and the shear rate.
- C) The force required to separate the disks axially is proportional to the separation rate, the square of the area, and inversely proportional to the initial separation cubed.

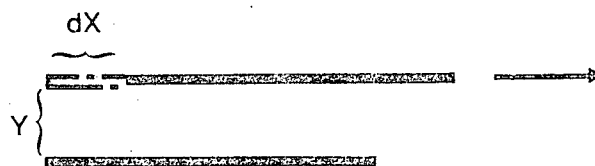
Figure 9.1

A



B

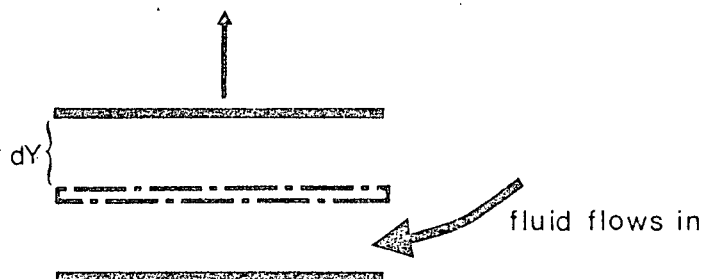
$$F = \frac{\pi R^2 \left[\frac{dX}{dt} \right] \eta}{Y}$$



$$\text{shear rate} = \left[\frac{dX}{dt} \right] / Y$$

C

$$F = \frac{1.5 \pi R^4 \eta \left[\frac{dY}{dt} \right]}{Y^3}$$



quite simply by applying the definition of viscosity presented in Chapter 4:

$$F = A \tau \left(\frac{dx}{dt} \right) y^{-1} = \pi R^2 \left(\frac{dx}{dt} \right) y^{-1}$$

where F is the shear force required to slide the discs apart at the velocity (dx/dt) , A is the area of the discs, and R the radius. By dividing the force by the disc area the stress necessary to move the discs at a given rate can be calculated:

$$\text{shear stress} = \tau \left(\frac{dx}{dt} \right) y^{-1}$$

The stress required to slide the plates apart thus depends directly on the rate at which they are separated and inversely on the thickness of the adhesive layer.

The force required to separate the discs axially has been calculated by Stefan (1874) as cited in Crisp (1973).

$$F = 1.5 \pi R^4 n \left(\frac{dy}{dt} \right) y^{-3}$$

where F is the axial force, and x , y and R are as before. Again this force can be expressed as a stress, (in this case tensile stress) by dividing by the area.

$$\text{Tensile stress} = 1.5 R^2 n \left(\frac{dy}{dt} \right) y^{-3}$$

The stress required to separate the plates axially is a function of the rate of separation as one might expect from a viscous fluid. However, the tensile stress is also highly

sensitive to the dimensions of the system, increasing as the square of radius and inverse cube of adhesive thickness. This dependence on the dimensions of the system is due to the geometry involved in pulling the discs apart. When two discs slide past each other no new fluid must be introduced between the plates. For the movement to occur axially, however, fluid must flow into the widening gap as the discs move apart. The larger the radius the greater the volume of fluid which must move in for a given separation, and the thinner the layer the smaller the "pipe" through which this fluid must be transported.

These equations may be used to provide an estimate of the adhesive ability of A. columbianus by using an average value for mucus in its fluid state of 40 poise and assuming an arbitrary separation rate of 1 mm/sec.

$$\text{Shear stress} = 400 \text{ N/m}^2$$

$$\text{tensile stress} = 6 \times 10^8 \text{ N/m}^2$$

It is evident that it is much easier to slide the two discs apart than to pull them apart axially and that the axial values can be quite large. In fact the axial value here would in reality be limited by the tensile strength of water, which has been estimated at $0.2 - 1.0 \times 10^8 \text{ N/m}^2$ (Hammel and Schollander, 1976) this explains at least on a qualitative basis the first two observations made on A. columbianus, that it adheres strongly to glass, and explains why the foot of a slug is not lifted when the animal is

crawling on a non-porous surface (Chapter 3).

The value for tensile stress calculated here is very large. If this estimate is correct it would require the application of a weight of 1.5×10^5 kg to detach a 20 gram slug. While no accurate figure is available for the adhesive strength of the slug's foot in tension, this calculated value is obviously many times too large. The reason for this discrepancy is two-fold:

1. The Stefan equation is calculated for discs immersed in a viscous fluid. Thus when the discs are separated more fluid is drawn in. For a slug anchored by fluid mucus to a glass plate the most likely fluid to be drawn in as the slug is pulled away from the substratum is air, the viscosity of which is roughly 1×10^{-5} that of mucus and will therefore contribute negligibly to adhesion. Thus the Stefan equation over estimates a slug's adhesive ability. The degree of overestimation cannot be determined without a clear understanding of the flow patterns of fluids beneath the slug's foot when a force is attempting to pull the foot away from the substratum. Data concerning these flow patterns are not yet available.

2. Any flaws (such as air bubbles in the mucus or dust on the glass surface) in the adhesive layer will form stress concentrations which will lower the effective adhesive strength of such a fluid system. Thus, while the system is in theory capable of tremendous adhesive strength, in reality the presence of unavoidable flaws will impose a much lower limit on adhesive strength. The theory of stress

concentrations will be discussed more fully later in this chapter.

Until the effects of these two factors are quantified the Stefan equation can only provide a very rough indication of the adhesive ability of the slug's foot. It is reasonably clear, however, that the simple presence of a viscous fluid beneath the foot provides the potential for a high adhesive strength.

Returning to the Stefan equation and remembering the assumptions used to calculate the values cited here, it will be seen that the high stress figure obtained is attributable to the relatively high rate of separation (coupled with the extremely thin layer of mucus). The lower the rate of separation the lower the stress. Thus a small stress applied over a long period of time will result in a large separation of the discs. This fact leads to another aspect of the adhesive system of slugs.

So far this discussion has assumed the presence of mucus in its fluid form. How does the situation differ for mucus in its solid form? Return to the situation shown in Figure 8.1c. In this case, however, the two discs are held together by a solid mucus layer 10 μm thick and a force applied to separate the discs at 1 mm/second (an initial tensile strain rate of 100/sec). At this rate the yield stress of A. columbianus pedal mucus in shear is about $1 \times 10^3 \text{ N/m}^2$. The stiffness of a material in tension is approximately 3 times that in shear so the yield strength of solid mucus (at this strain rate) is about $3 \times 10^3 \text{ N/m}^2$; a

value much lower than that calculated by the Stefan equation. However this is only the stress required to change the solid mucus into a fluid. Once the mucus behaves as a fluid the arguments outlined above concerning the Stefan equation apply. Thus if the slug is adhering with solid mucus, the problem of its ultimate adhesive strength in resisting rapid deformation returns in the end to the question of the adhesive strength of a viscous fluid.

The solidity of mucus is, however, an important factor in resisting smaller forces on the time scale (approximately 1 second) imposed during locomotion as has been thoroughly discussed in Chapter 7. The solidity of mucus will also allow it to function as an effective adhesive against the force of gravity on the time scale of minutes and hours. On this time scale a fluid adhesive would be ineffective. The properties of mucus and the mucus' effectiveness as an adhesive at large times have already been discussed (see Chapter 4).

In light of this discussion it seems reasonable to assume that pedal mucus forms an effective adhesive. If this is so how does the slug ever manage to detach part or all of its foot from the substratum? When feeding, slugs are often seen to lift the anterior $1/4$ to $1/3$ of the body in order to reach a leaf. Also, if pedal mucus is such a strong adhesive how is it possible to so easily peel a slug from a piece of glass?

The answer to these questions lies in the formation of stress concentrations in the mucus. Take for example the

situation depicted in Figure 9.2. A tensile stress is placed on a block of material. It can be imagined that the force acting on one infinitesimal area on the top of the block can be traced, molecule to molecule, through the material to an area on the bottom of the block. Thus the distribution of forces within the block can be visualized as a set of stress trajectories as shown in Figure 9.2a. If the force is evenly distributed over the top and bottom surfaces of the block the stress trajectories will be parallel and evenly spaced and the force will be spread uniformly throughout the material. Now if a crack is introduced into one edge of the material the situation is changed. The force acting on the material above the crack can no longer be passed directly to the material below the crack. Instead the stress trajectories must curve around the end of the crack and in doing so squeeze together. Thus all the force that would normally be applied around the area of the crack is concentrated in the area at the crack tip. The increase in stress at the crack tip can be quite large and is expressed by

$$S_t = S (1 + (2l/w)) \quad (\text{Wainright et al, 1976; Gordon, 1972})$$

where S_t is the stress at the crack tip, S is the overall stress, and l and w are the dimensions of the crack as shown in Figure 9.2b. This equation can be applied directly to adhesive structure of a slug (Figure 9.2c). The edge of the slug's foot provides a ready made crack. As a reasonable

FIGURE 9.2. Stress concentrations.

- A) In a uniformly loaded sample the stress trajectories are uniformly spaced.
- B) A crack causes the trajectories to converge, forming a stress concentration.
- C) The edge of a slug's foot will act as a crack, causing a stress concentration.

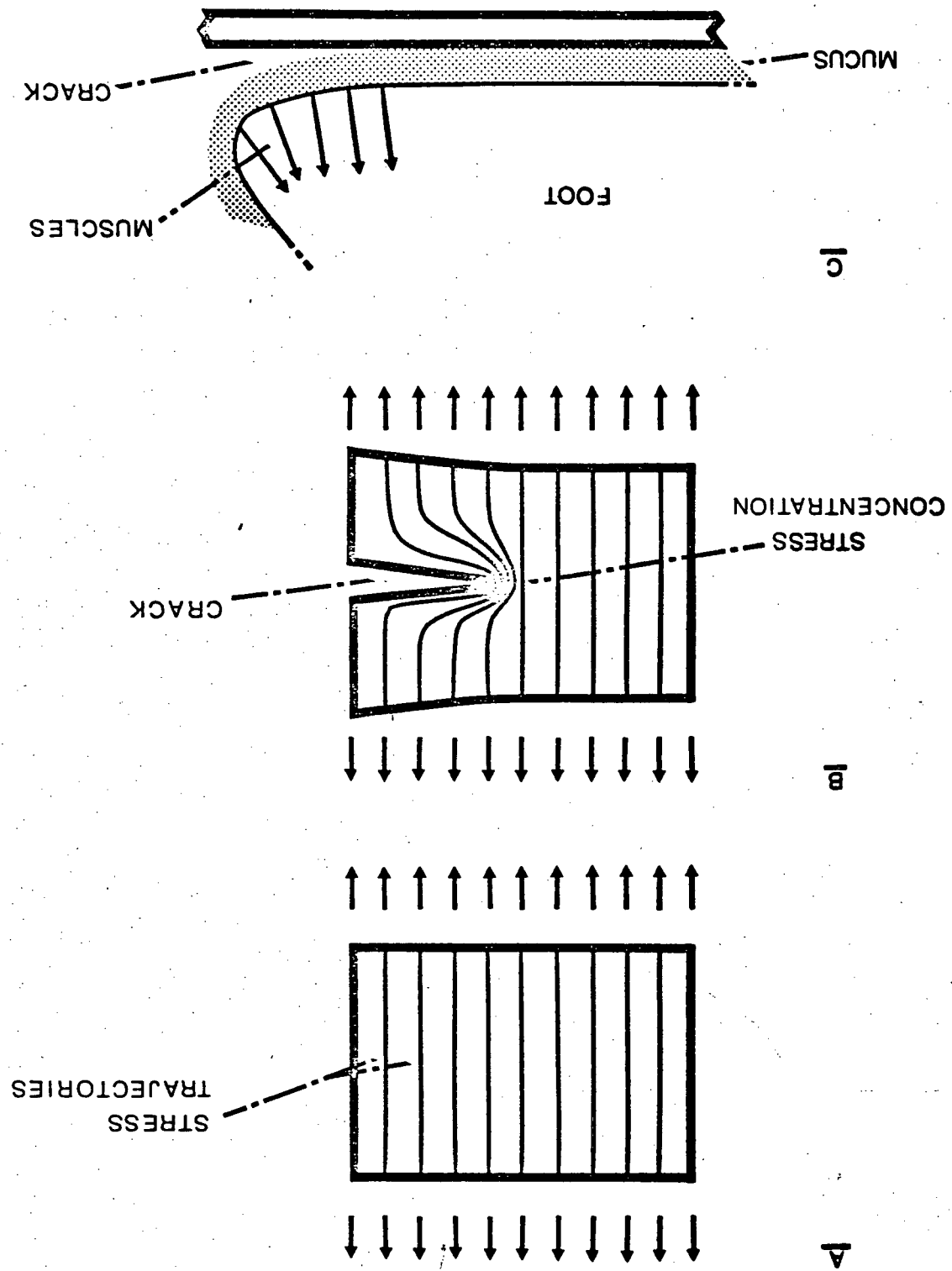


Figure 9.2

example set the "crack length" at 100um. The crack width cannot be any larger than the thickness of the mucus layer and is likely to be considerably smaller, for example set w conservatively at 5 um. Thus the stress concentration

$$S/St = (1 + 200/5) = 41$$

Any stress applied to the foot edge by the contraction of muscle will be magnified 41 times at the crack tip. As the crack extends the stress concentrations will grow larger and it will be easier to detach additional areas of the foot. In this manner the slug, by applying a relatively small force at its foot edge, can detach its foot from the substratum.

In order to take advantage of the crack present at the edge of the foot the slug contracts muscles attached to the edges. However when an inquisitive zoologist attempts to grasp a slug and pull it off a piece of glass he usually pulls the slug from the center of its back in which case very little force is applied directly to the foot fringes and the edge cracks are not propagated. If however an edge of the foot is pried up and pulled upon a large crack is created, a large force can be applied to that crack area and the slug is easily peeled off as the crack propagates. This presence of stress concentration at crack tips can explain the third observation noted at the beginning of this chapter.

As already mentioned the existence of other cracks in

the mucus layer, such as air bubbles trapped as the slug moves, cavities in the substratum, or dirt particles will form stress concentrations. The presence of a number of these stress concentrations would lower the adhesive strength of the mucus layer to a value well below its theoretical maximum.

A TEST

As mentioned above the structure of a slug's body does not lend itself to the measurement of adhesive strength. In one case, however, it proved possible to measure the adhesive strength of slugs.

If a mass is swung at the end of a string the path taken by the mass is the result of two factors:

1. The mass is travelling at a certain velocity. If the string were not present the mass would travel in a straight line.

2. In order for the mass to travel in a circular path a constant radial acceleration must be applied to the mass. This radial acceleration leads to the "centrifigal force", F .

$$F = ma$$

where m is mass and a is acceleration. The acceleration is calculated as

$$a = w^2r$$

where w is the angular velocity of the mass as it travels in its circular path and r is the radius of the circle. Thus

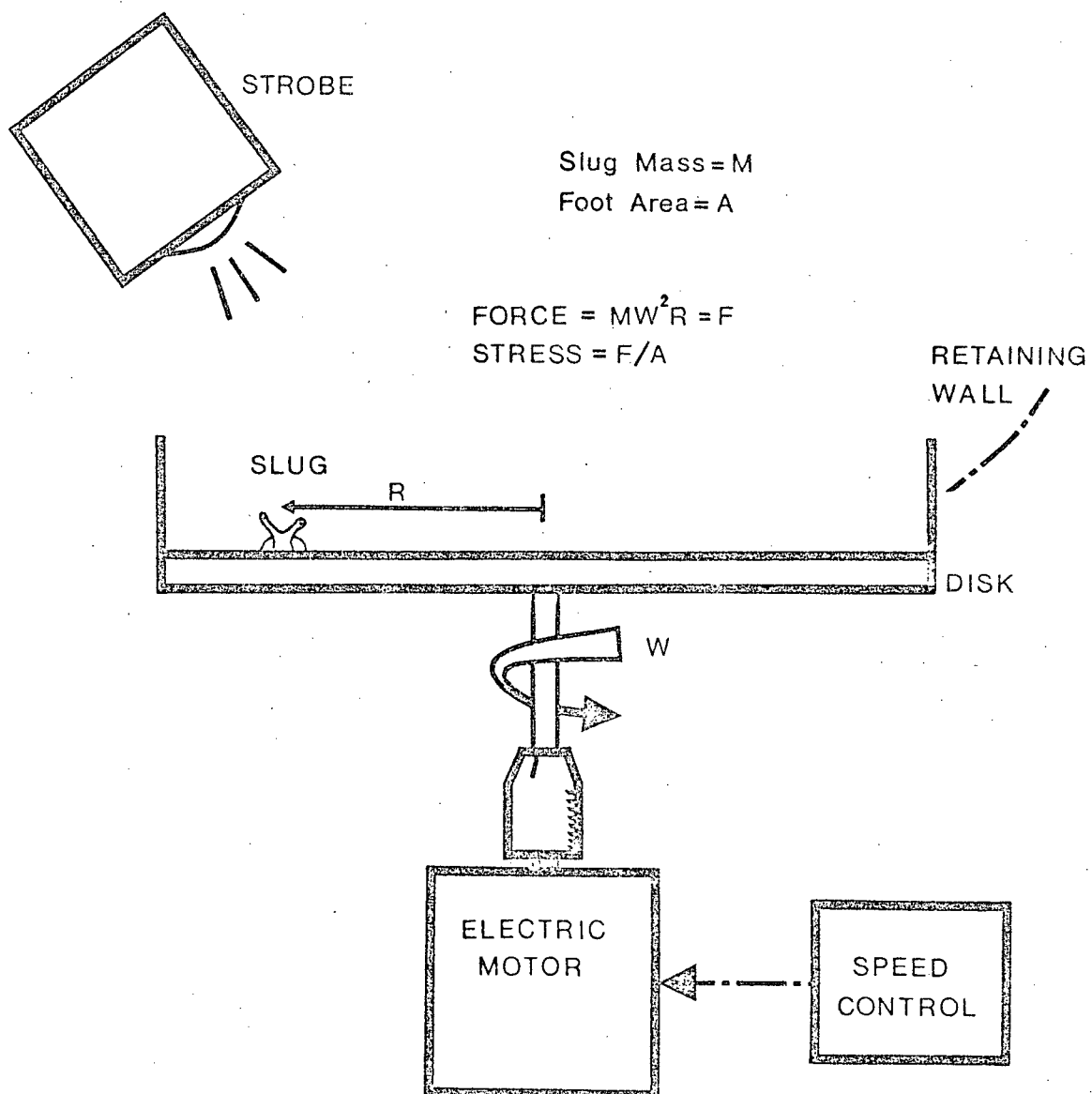
$$F = mw^2r$$

If a slug is placed on a disk, allowed to adhere, and the disk is then rotated, a centrifugal force will be exerted on the slug. As long as the slug remains stationary relative to the disk this centrifugal force must be resisted by the solid adhesive mucus beneath the slug. When the disk is rotated at sufficient speed, the slug will be forced outwards on the disk. Thus if the mass of the slug and its foot area, the radius at which the slug is placed on the disk and the angular velocity required to move the slug are all known the adhesive shear strength of the mucus can be calculated.

This experiment was carried out using the apparatus shown in Figure 9.3 . The foot area of a small A. columbianus (0.18 - 0.26 grams) was measured and the slug then placed on the disk at a measured radius and allowed to adhere. The disk was rotated by a Cole-Parmer Master Servodyne motor, and the frequency of revolution matched manually with a calibrated stroboscope. The rate of rotation was increased until the slug's adhesive yielded and the slug was spun outwards to the retaining wall. The rate of rotation was then read off the stroboscope and the yield stress calculated. Three to five trials were carried out on each of five slugs and the average yield stress calculated was 780 (+- 335) N/m² . It was not possible with this

FIGURE 9.3. A device for measuring the shear strength of Ariolimax columbianus pedal mucus under the slug. The electric motor spins the disk at an angular velocity, W , that is measured with the stroboscope. The force acting to slide the slug radially outwards is MW^2R , where M is the slug's mass, and R is the radius. In this manner the force (and the stress) at which the mucus yields and the slug begins to slide, can be measured.

Figure 9.3



apparatus to measure the shear rate incurred by centrifugation. However, since the rate of rotation was increased slowly the centrifugal force was applied over a number of seconds. Consequently the shear rate should be quite low. In Chapter 4 the yield stress of A. columbianus pedal mucus at a shear rate of approximately 5/second (the lowest measured) was 320 N/m^2 . Thus given the crude nature of this experiment the predicted and measured values match quite closely.

CHAPTER TEN

Conclusions

This study has examined in considerable detail the mechanism of adhesive locomotion found in one species of slug, Ariolimax columbianus. Is the mechanism described here unique to this one species or can it be generalized to account for the locomotion of other types of gastropods?

Before dealing directly with this question it will be useful to review the fundamental requirements for adhesive locomotion. Any gastropod utilizing muscular pedal waves will have two types of areas present on the foot sole during movement: 1. areas that are moving forward, and 2. areas that are stationary. The moving segments of the foot must encounter some amount of sliding friction as they slide across the substratum. The force required to overcome this friction is resisted by the areas of the foot which are stationary. This resistance is brought about by an interaction of the stationary portions of the foot with the substratum beneath them. Thus, if the resistance of the stationary portions of the foot to being moved backwards is greater than the resistance of the moving portions of the foot to being moved forwards the animal will be able to crawl. This inequality of forward and backwards resistances can be brought about in any of three ways:

1. If the resistance to movement per unit area is the same for all parts of the foot, it is necessary that a smaller area be moved forwards than remains stationary.

2. The resistance to movement per unit area can vary from one portion of the foot to the next such that the moving areas of the foot, taken as a whole, show less resistance than the stationary portions. In this case the moving segments of the foot could have a larger area than the stationary segments provided that the resistance to movement was sufficiently smaller under the moving areas.

3. Both 1. and 2. could be acting simultaneously.

It has been shown in this study that A. columbianus is a good example of this third case. Direct waves (as used by A. columbianus) start at the posterior end of the animal by compressing the foot. Areas of forward movement (the waves) are thus necessarily smaller than the stationary areas. In addition, the physical properties of the pedal mucus are such that the forward sliding resistance is considerably smaller than the backwards resistance. The result is an effective locomotory system. We now return to the original question of this chapter; could this, or a similar mechanism, be operating in other gastropod species? The various species will be discussed according to their locomotory types (as defined in Chapter 3).

Direct Monotaxic Waves

The prominent members of this group are the terrestrial slugs and snails. As regards the mechanism of locomotion the vast majority of these are very similar in all respects to A. columbianus. Consequently, I see no reason why the model proposed here for A. columbianus cannot be applied

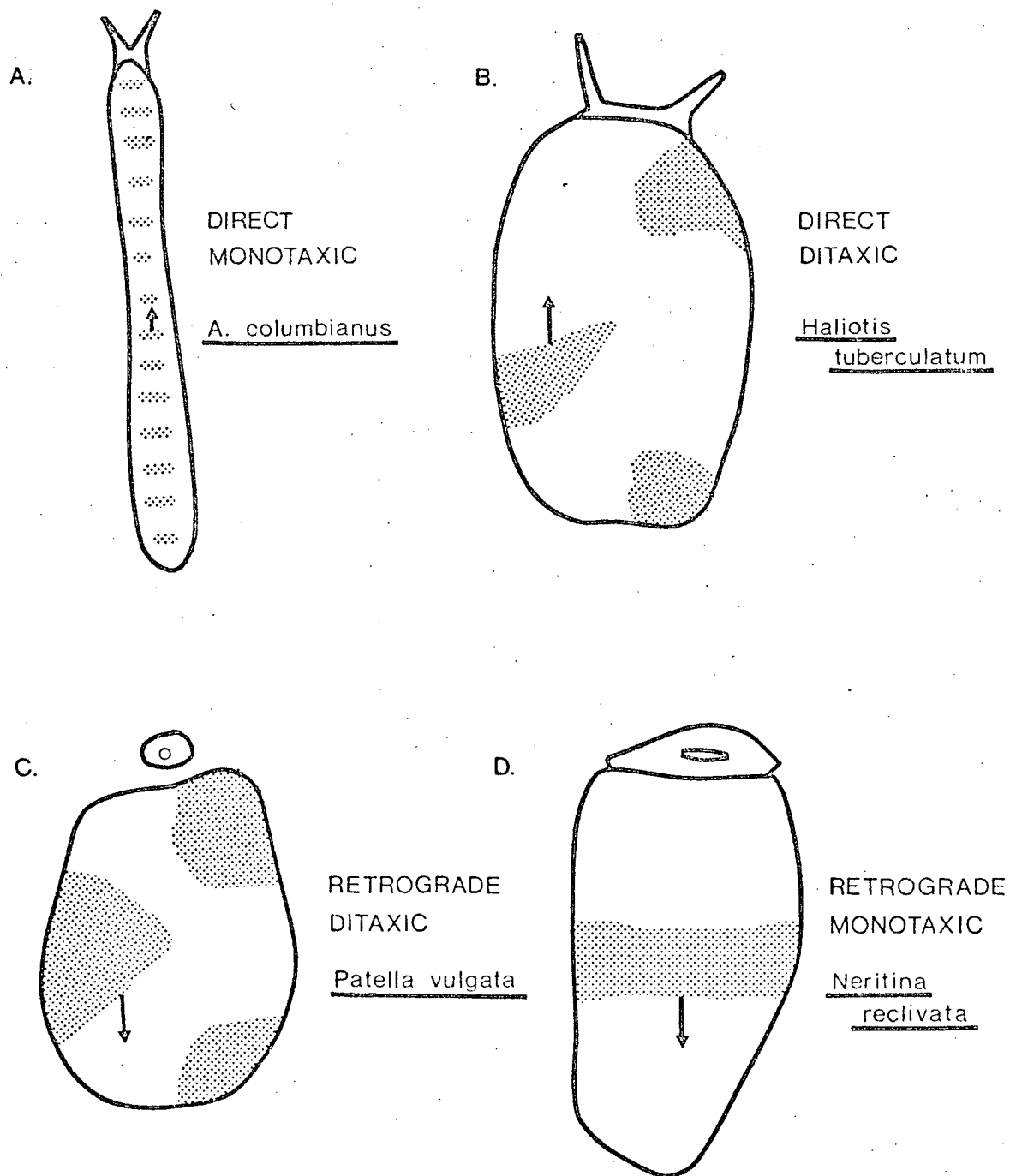
directly to these other species.

Direct Ditaxic Waves

Direct ditaxic waves (see Figure 10.1b) are found in relatively few species of gastropods such as the abalones Haliotidae and some species of Thais. The best description of ditaxic direct waves is that of Lissman (1945a) using Haliotis tuberculata, and this study is used as the basis for this discussion. As with direct monotaxic waves, the areas of forward motion in Haliotis are compressed relative to the stationary areas so that locomotion could occur even without a variation in frictional resistance under different areas of the foot. The abalone does, however, exhibit a mechanism for lowering the resistance to movement under the moving segments of the foot. In these animals the foot can clearly be seen to lift during the passage of a wave. This process is facilitated by the position of the waves on the foot. Unlike the slug, where the waves are in the center of the foot and enclosed by a rim, the abalone's wave moves along the edges of the foot and are thus directly in contact with the surrounding fluid. It seems likely that the water being sheared under the lifted portion of the foot will offer less resistance to movement than the mucus under the stationary portions of the foot. Thus, at least in the case of the abalone, animals utilizing direct ditaxic waves employ a different mechanism than that proposed for A columbianus. The end result is similar but the requirement for specialized mucus properties is absent.

FIGURE 10.1. A diagrammatic representation of the four regular forms of pedal waves. The stippled areas represent those areas on the foot which are moving, and the arrows indicate the direction of movement.

FIGURE 10.1



Retrograde Ditaxic Waves

Retrograde ditaxic waves (see Figure 10.1c) are the most common form of pedal wave found among prosobranch gastropods (Miller, 1974b). Jones and Truman (1970) have studied the locomotion of the limpet Patella vulgata, and their study will form the basis of this discussion. Retrograde waves, in contrast to direct waves, are initiated at the anterior end of the foot as the foot is extended forwards. The wave of extension is then passed posteriorly along the foot. The length of a single wave is typically one third of the foot length or greater (Miller, 1974a). It seems likely that the area of these large waves of extension is larger than the area of the stationary portions of the foot. Consequently these animals must possess a mechanism for lowering the forward sliding resistance relative to the backwards. Jones and Trueman (1970) propose that the foot is lifted during movement and that the space filled with water, as with abalones. Unlike abalones, however, the lifting of the foot is not visually apparent. Jones and Trueman (1970) based their conclusions on measurements made as a limpet crawled over a hole in a glass plate. It has been pointed out in Chapter 3 that measurements of foot lifiting made in this manner may not conform to the situation when an animal is crawling over a solid, inflexible surface. It is highly probable, then, that limpets (and presumably other prosobranchs) do not lift their foot during locomotion and must therefore rely on the properties of their pedal mucus to affect movement. The

resolution of this question awaits further study.

Retrograde Monotaxic Waves

While retrograde monotaxic waves are fairly common in gastropods (Miller, 1974b) they have received relatively little attention. The most extensive study to date is that of Gainey (1976) dealing with Neratina reclinata. As with ditaxic retrograde waves, monotaxic retrograde waves are waves of extension (see Figure 10.1d). Each wave is typically one third of the foot length or greater and one or two waves are present on the foot at any one time (Miller, 1974a). In N. reclinata only one wave is present on the foot. As a consequence the area of the moving segment of the foot is less than that of the stationary segments. It is not known whether this is typical of this type of wave. If indeed the stationary area exceeds the moving area, no reduction in resistance beneath the moving area need necessarily exist. A reduction in resistance beneath the waves would however be advantageous. As with other wave forms, a lowering of resistance could be brought about either by a lifting of the foot or the properties of the pedal mucus. To my knowledge no measurements have been made concerning this problem. Thus, until more data have been gathered, the possible existence in these animals of a mechanism similar to that in A. columbianus cannot be determined.

Summary

In summary, the mechanism of adhesive locomotion proposed in this study is likely to apply to terrestrial slugs and snails. It is possible that it may apply to other forms of pedal locomotion but until further information is known about these forms of locomotion this possibility remains uncertain.

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